

LOCALIZATION OF HISTONE GENES AND
NUCLEOLUS ORGANIZING REGIONS
IN THE POLYTENE AND MITOTIC
KARYOTYPES OF **DROSOPHILA AFFINIS**
AND **DROSOPHILA SUBOBSCURA**

INAUGURAL-DISSERTATION
ZUR
ERLANGUNG DER PHILOSOPHISCHEN DOKTORWÜRDE

VORGELEGT DER
PHILOSOPHISCHEN FAKULTÄT II
DER
UNIVERSITÄT ZÜRICH

VON
CLAUDIA RENATE SCHWEIZER-HESS
VON GLARUS

BEGUTACHTET VON
FRAU PD Dr. E. HAUSCHTECK-JUNGEN
HERRN PROF. Dr. M. BIRNSTIEL

ZÜRICH 1979

1981



Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41507206_0024

**LOCALIZATION OF HISTONE GENES AND
NUCLEOLUS ORGANIZING REGIONS
IN THE POLYTENE AND MITOTIC
KARYOTYPES OF DROSOPHILA AFFINIS
AND DROSOPHILA SUBOBSCURA**

INAUGURAL-DISSERTATION
ZUR
ERLANGUNG DER PHILOSOPHISCHEN DOKTORWÜRDE

VORGELEGT DER
PHILOSOPHISCHEN FAKULTÄT II
DER
UNIVERSITÄT ZÜRICH

VON
CLAUDIA RENATE SCHWEIZER-HESS
VON GLARUS

BEGUTACHTET VON
FRAU PD Dr. E. HAUSCHTECK-JUNGEN
HERRN PROF. Dr. M. BIRNSTIEL

ZÜRICH 1979



DEM ANDENKEN MEINER MUTTER

UND

MEINEM VATER

CONTENTS

I. INTRODUCTION	1
II. MATERIAL AND METHODS	4
II.1. Material	4
II.2. Methods	5
II.2.1. Breeding of Flies	5
II.2.2. Cytological Preparation of Salivary Gland Chromosomes	5
II.2.3. Cytological Preparation of Mitotic Chromosomes From Larval Neuroblasts	6
II.2.4. Analysis of the Polytene Karyotype of <u>D. affinis</u>	6
II.2.5. Isolation of Cytoplasmic ^3H -18+28S rRNA	7
II.2.6. Nick-Translation of Histone Genes	9
II.2.7. In Situ Hybridisation Experiments	10
II.2.8. Silver-Staining of Polytene and Mitotic Chromosomes	13
II.2.9. Giemsa C-Staining of Mitotic Chromosomes	14
III. RESULTS	15
III.1. Analysis of the Polytene Karyotype of <u>D. affinis</u>	15
III.1.1. Chromosome B	16
III.1.2. Chromosome C	18
III.1.3. Chromosome E	19

III.1.4. Chromosome F	20
III.1.5. Chromosome X	20
III.2. In Situ Hybridisation Experiments With ³ H-18+28S rRNA	37
III.2.1. In Situ Hybridisation to Polytene Nuclei of <u>D. affinis</u> and <u>D. subobscura</u>	37
III.2.2. In Situ Hybridisation to Diploid Nuclei From Larval Brain Ganglia of <u>D. affinis</u> and <u>D. subobscura</u>	39
III.3. Silver-Staining of Polytene and Diploid Nuclei in <u>D. affinis</u> and <u>D. subobscura</u>	41
III.3.1. Polytene Nuclei	42
III.3.2. Diploid Nuclei	44
III.4. In Situ Hybridisation of Cloned Histone Genes	48
III.4.1. In Situ Hybridisation to the Polytene Karyotype of <u>D. affinis</u>	48
III.4.2. In Situ Hybridisation to the Polytene Karyotype of <u>D. subobscura</u>	49
IV. DISCUSSION	52
IV.1. General Discussion	52
IV.2. Analysis of the Polytene Karyotype of <u>D. affinis</u>	54
IV.3. Localization of Nucleolus Organizing Regions (NORs) in <u>D. affinis</u> and <u>D. subobscura</u>	57
IV.4. Localization of Histone Genes in the Polytene Karyotypes of <u>D. affinis</u> and <u>D. subobscura</u>	62

V. REFERENCES	68
VI. FIGURE LEGENDS	81
VII. FIGURES	92
VIII. SUMMARY	109
Abbreviations	111
Acknowledgements	113
Curriculum vitae	114

I. INTRODUCTION

Drosophila affinis and Drosophila subobscura share a genetic phenomenon widely spread within the genus - an inversion polymorphism involving all chromosomes within the karyotype of the relevant species (Stone, 1962; Goetz, 1965, 1967; Jungen, 1968a). Combinations of X-linked inversions, namely $A_{2+3+5+7}$ in Drosophila subobscura (Jungen, 1968b), or XS-I combined with XL-II, or XS-I combined with XL-IV in Drosophila affinis (Voelker, 1972), are particularly interesting since they are associated with a sex ratio phenomenon, a so called meiotic drive system: male carriers of these inverted X-chromosomes produce in both species a vast majority of daughters in their progeny. The expressivity of the phenomenon does not depend on the mother's karyotype.

Light microscopical examination of spermatogenesis in sex ratio males showed degeneration of a variable number of spermatids per cyst ranging from 0 to 100% (Hauschteck-Jungen and Maurer, 1976). Electron microscopical studies of cysts from sex ratio males indicated abnormal arrangements of cell organelles, such as the axoneme, the mitochondrial derivatives, the acrosome and the nucleus in the spermatids (Dubler-Hänggi, 1977). Presumably, the aberrant spermatids are carrying the Y-chromosome. But since in many cysts more than half or even all of the spermatids degenerate, the effects are not limited to the Y-bearing spermatids. This raises the possibility that some of the fundamental processes of spermatogenesis may be affected. One of these processes is the appearance of an arginine rich histone, which performs an important role in the final condensation of chromatin into the dense and compact mass typical for the mature sperm. It has been proposed for the Segre-

gation Distorter (SD) meiotic drive system in Drosophila melanogaster that the SD-chromosome may affect the synthesis and/or utilization of histone (Tokuyasu et al., 1977), since chromatin condensation is aberrant in spermatids of SD males. Additional support for assuming a correlation between Segregation Distorter and the histone loci in D. melanogaster came from the mapping of SD near the histone genes. Up to now, the position of the histone genes is only known for D. melanogaster.

In meiotic drive systems the disturbance of cell differentiation is restricted to gametogenesis. Ribosomal genes may be involved, especially in the sex ratio meiotic drive systems which depend on the paternal X-chromosome. In all Drosophila species so far analysed, ribosomal genes were found on the X-chromosomes.

The present studies ask, whether histone genes and 18+28S rRNA genes are localized on the X-chromosomes in Drosophila affinis and Drosophila subobscura. For cytogenetical investigation of these questions, polytene and mitotic chromosomes were used, the former facilitating exact localization of the relevant genes, and the latter giving also information on possible location of these genes on the Y-chromosome which does not polytenize in salivary glands.

My studies require a precise knowledge of the polytene karyotype. In spite of the published observations on different gene arrangements in the Drosophila affinis subgroup (Miller, 1977), no cytological map of

the polytene karyotype of Drosophila affinis has been published so far. Therefore, an analysis of the salivary gland chromosomes was carried out in the first part of this work.

II. MATERIAL AND METHODS

II.1. Material

To analyse the polytene karyotype of Drosophila affinis, cytological preparations of salivary gland chromosomes from late third instar larvae and prepupae of wild stocks were used. Each stock was obtained by inbreeding the offspring of a single parental couple, that had been caught in Spartenburg (North Carolina) in 1975 (Hauschteck-Jungen, personal communication).

In situ hybridisation of 18+28S rRNA and silver staining were performed on larval neuroblasts and on polytene nuclei of prepupal salivary glands from wild stocks of D. affinis and D. subobscura (Küsnacht and Tunis 2). In both species, individuals carrying inverted or standard karyotypes were examined.

Cloned histone genes (see II.2.6.) were hybridised in situ to standard polytene chromosomes of prepupal salivary glands from D. affinis and D. subobscura (Küsnacht).

In the in situ hybridisation- and silver staining experiments, prepupal salivary glands and larval neuroblasts of D. melanogaster wild stocks were included in each experimental series as control. These stocks had been collected in 1976 near Zürich and have been cultivated since then in our laboratory.

II.2. Methods

II.2.1. Breeding of Flies

The flies were cultivated in 150 cm³ bottles on a maize/agar medium, composed of 80 gr cane sugar, 150 gr maize, 12 gr agar, 10 gr dried yeast and 0.25 gr "Nipagin" (p-hydroxibenzoic acid methyl ester) in 1000ml water. The medium was inoculated with approx. 0.2 ml (per bottle) fresh baker's yeast, stirred up in water. The breeding temperature varied between 16⁰ and 22⁰C. The culture medium was renewed once per week.

II.2.2. Cytological Preparation of Salivary Gland Chromosomes

Salivary glands that were used in the experiments mentioned above (see II.1.), were excised from larvae and prepupae of D. affinis, D. subobscura and D. melanogaster in a buffered insect Ringer solution (pH 7). prepupal salivary glands were recognized in virtue of their swollen appearance, which is absent in larval salivary glands. The salivary glands were treated according to the technique suggested by Atherton and Gall (1972): they were transferred into ethanol/acetic acid (3:1) and were left for fixation for 2-3 min. They were then incubated in a drop of 50% acetic acid on an ethyl ether cleaned and gelatinized slide for approx. 20-30 sec. Eventually, they were covered with a siliconized cover-slip. Optimal distribution of the polytene chromosomes on the slide was obtained by slightly tapping upon the cover-slip with a thin knitting needle. Hereafter, the slide was kept on a warm plate for not longer than 1 min. at 45⁰-47⁰C, in order to maintain optimal appearance of the chromosome's morphology. Slides were frozen on

dry ice, and subsequently, the cover-slips were removed from the slides with a razor blade. The slides were immediately transferred into 96% non-denatured ethanol for at least 5 min. and were then air-dried.

The preparations, that were used for karyotype analysis in D. affinis, were stained in a drop of 2% orcein (Merck)/50% acetic acid/lactic acid (1:1) for 2-5 min. The slides were then rinsed in distilled water, air-dried and finally sealed in Euparal.

II.2.3. Cytological Preparation of Mitotic Chromosomes From Larval Neuroblasts

Brain ganglia from late third instar of D. affinis, D. subobscura and D. melanogaster were dissected in buffered insect Ringer solution. They were then hypotonically treated by incubation in 1% sodium citrate for 30-60 min. Subsequently, they were fixed in a drop of 50% acetic acid for a few seconds, and were squashed under a siliconized cover-slip. The slides were frozen on dry ice. After removal of cover-slips, they were immediately incubated in 96% non-denatured ethanol for at least 5 min. The air-dried slides were stored until further use (see p. 14).

II.2.4. Analysis of the Polytene Karyotype of D. affinis

Well spread polytene chromosomes in optimal conservation of their band morphology were photographed with a high contrast film (Agfaortho-25). Prints were made, on which the total length of chromosome arms as well as the relative position of landmarks were determined, employing the watch cog-wheel method described by Baumberger (1970) for chro-

mosome measuring. The watch cog-wheel, with a constant distance between the cogs, was run along the print of the photographed chromosome arm, thus generating marks, where the cogs had been pressed into the print. The number of intervals (units) between the proximal end of a particular chromosome arm and a particular landmark band on this arm (l) was divided by the total number of units along the chromosome arm (L). The relative position of the landmark was determined by the ratio $\frac{l}{L}$. The relative length of the chromosome arm was calculated by dividing the total length of any particular chromosome arm (L_x) by the total length of the short arm of chromosome B (L_{Bs}), $\frac{L_x}{L_{Bs}}$, at a constant magnification index.

II.2.5. Isolation of Cytoplasmic ^3H -18+28S rRNA

2×10^6 cells from an Echaliér cell line of D. melanogaster were radioactively labelled in culture by adding 10 mC ^3H -uridine (spec. act. 29C/mM) to 1 ml of Schneider's culture medium for four days. The cells were then centrifuged, and the Schneider medium was removed. The cells were repeatedly washed in tricine buffered sterile Ringer's solution, and were centrifuged each time for 5 min. at 3500 rpm at 4°C in the swing-out rotor of the Sorvall RC-2B centrifuge. Hereafter, the cell membranes were disrupted by treating the cells in sterile lysis buffer, which consisted of 100 mM NaCl, 10 mM CaCl_2 , 30 mM Tris (pH 8.3), 35 $\mu\text{g/ml}$ spermine, 25 $\mu\text{g/ml}$ PVS and 0.5% Baycovin. 0.2% NP40 was added to this mixture. The cells were light-microscopically checked at intervals, until most of the intact cell nuclei were delivered from cytoplasm. They were centrifuged for 3 min. at 5000 rpm, then EDTA (pH 7.8) was added to the aqueous phase to a final concentration of 25 mM.

the cytoplasmic RNA was extracted by adding an equal volume of a water saturated phenol/chloroform mixture to the aqueous phase. This mixture was intensively shaken and centrifuged at 8000 rpm for 5-10 min. at 4°C. The upper phase was removed, and the phenol extraction was repeated twice. Three chloroform extractions followed, each time adding an equal volume of chloroform/isoamylalcohol (24:1, v/v) to the aqueous phase with subsequent centrifugation in the manner stated above. A sterile NaCl solution was finally added to the upper phase to a concentration of 300 mM. The RNA was precipitated by the addition of three volumes of absolute ethanol, and was stored at -20°C.

The precipitated RNA was centrifuged for 5-10 min. at 8000 rpm, and the dry pellet was dissolved in 5-10 ml of a buffer, containing 500 mM NaCl, 50 mM Tris (pH 7.5) and 0.5% SDS. This probe was repeatedly run through an oligo-dT column, in order to remove the portion of polyA⁺ mRNA. The amount of the remaining cytoplasmic RNA was determined by measuring its absorbance at A₂₆₀. This RNA was ethanol-precipitated at -20°C.

For separation of RNA fractions in a sucrose density gradient, the RNA pellet was dissolved in 400 µl of a buffer, containing 100 mM NaCl, 10 mM Tris (pH 8.5), 1 mM EDTA (pH 7.5) and 0.5% SDS. Separation of the RNA probe was performed on a linear 15-30% sucrose density gradient. 200 µl of the probe were loaded onto each of an RNAase free buffered 6.8 ml gradient, and were centrifuged to equilibrium in a SW40 rotor (Spinco L2-65B) at 30000 rpm at 23°C for 20 hrs. Fractions of 0.5 ml were collected. The radioactivity of the 18S+28S rRNA fractions was determined by scintillation counting after 5%TCA precipitation on GF-filter paper. The relevant fractions were pooled, and ammonium acetate was added to a final concentration of 300 mM prior to ethanol precipitation

at -20°C .

A total of 4.08×10^8 cpm (spec. act. 1.2×10^6 cpm/ μg) was recovered at a scintillation counter efficiency of approx. 30%.

II.2.6. Nick-Translation of Histone Genes

A histone gene insertion from D. melanogaster (5 kbp) within an E. coli plasmid (13 kbp) was used as template (pDmh 604) in nick-translation reaction. The DNA fragment inserted into plasmid pDmh 604 codes for each of the five different histone mRNAs (Burckhardt, unpublished). Tri-³H-deoxyribonucleotide triphosphates (Radiochemical Centre Amersham) were obtained as ammonium salts in 50% ethanol at a concentration of 1 mC/ml. The specific activities were: 8-³H-dATP 29, methyl-³H-TTP 30, 5-³H-dCTP 22, 8-³H-dGTP 10.2 C/mM. E. coli DNA polymerase I (500 units in 0.1 ml) was purchased from Boehringer Mannheim GmbH.

10^{-2} mC each of all ³H-deoxyribonucleotide triphosphates were mixed in a small test tube. 0.3 μg of pDmh 604 DNA (in 10 mM Tris-HCl, pH 7.5) was added. When this mixture had been dried in vacuum, the dry pellet was dissolved in 2 μl of a 5 x salt buffer solution, which contained 250 mM Tris-HCl, 5 mM dithiothreitol (DTT), 1.5 mg/ml bovine serum albumine and 50 mM MgCl_2 , pH 7.5. Distilled water was added up to a volume of 4.9 μl , followed by the addition of 3.1 μl of pancreatic DNAase I (Worthington) at 10^{-5} mg/ml and 2 μl (10 units) of E. coli DNA polymerase I. The reaction mixture (0.01 ml) was incubated at 14°C for 2 hrs. The reaction was then stopped by the addition of 0.01 ml 10% SDS. 0.5 mg E. coli 4S DNA in 10 mM Tris-HCl was added as carrier. By the addition of 0.2 ml distilled water the mixture was brought to a final volume of 0.3 ml and

was then incubated at 37°C. for 15 min.

The 0.3 ml mixture containing the reaction product was run through a Sephadex-G50 column. For each fraction, absorbance at A_{260} was measured. The specific activity of the reaction product was 1.7×10^7 cpm/ μ g. A total of 5×10^6 cpm was collected. The labelled pDmh 604 DNA was recovered by ethanol precipitation in the presence of E. coli 4S carrier DNA.

II.2.7. In Situ Hybridisation Experiments

a) Preparation of Slides

In each experiment, a series of slides was hybridised, which included three or four squash preparations of prepupal salivary glands and larval brain ganglia respectively from D. affinis and D. subobscura as well as one from D. melanogaster as control. The slides were prepared for in situ hybridisation according to the method of Pardue and Gall (1975). Each cytological preparation (see II.2.2. and II.2.3.) was covered with 100-150 μ l of ribonuclease from bovine pancreas (Boehringer Mannheim GmbH), which was used at a concentration of 0.2 mg/ml in 2 x SSC at 37°C for 1 hr. The pancreatic ribonuclease was then removed by several rinses of the slides in 2 x SSC. After incubation of the slides in 70% non-denatured ethanol three times and twice in 96% ethanol, each time for 5 min., they were air-dried.

Denaturation of chromosomal DNA was performed by incubating the preparations in 0.07N NaOH. The duration of the treatment slightly varied, depending on the room temperature, ranging between 3 and 5 min. In each series one control slide was excluded from DNA denaturation. After several rinses in 70% and 96% ethanol, the slides were air-dried and rea-

dy for in situ hybridisation.

b) In Situ Hybridisation of ^3H -18+28S rRNA

The precipitated probe of ^3H -18+28S rRNA was centrifuged for 10-15 min. at 8000 rpm, the pellet was dissolved in 2 x SSC at a concentration of 34 $\mu\text{g}/\text{ml}$, and the probe was stored at -20°C . Each slide carrying polytene chromosomes of one of the three Drosophila species was provided with 10^5 cpm in 25 μl 2 x SSC, and was covered with a siliconized cover-slip. All cover-slips were boiled in 1N HCl for 3 min., subsequently rinsed in deionized water, and sterilised before use.

Squash preparations of larval brain ganglia were incubated in 1.7×10^5 cpm in 25 μl per slide. The incubation mixture comprised E. coli rRNA at a concentration of 25 $\mu\text{g}/\text{ml}$ as carrier. All slides were incubated at 67°C for 14-18 hrs. in moist chambers consisting of Petri-dishes of 10 cm in diameter each. The bottom of each Petri-dish was covered with a filter paper moistured with 10 ml of 2 x SSC. Two rubber rings were placed on the filter paper, which held the slide in a fixed position. The Petri-dishes were sealed with Parafilm, in order to avoid evaporation of the SSC-solution and consequently drying out of the chambers in the course of incubation.

c) Preparation of Incubation Mixture For In Situ Hybridisation of Histone Genes

40 μl of nick-translated DNA, dissolved in deionized water, was denatured by adding 4 μl 1N NaOH and incubating for 15-20 min. at $20-21^{\circ}\text{C}$. An ice cold mixture of 4 μl 1N HCl, 33 μl 20 x SSC and 80 μl formamide was added. This mixture was made up immediately before use.

d) In Situ Hybridisation of Nick-Translated Histone Genes

The technical details were the same as those stated in section II.2.7.b). 20 μ l of the incubation mixture, containing 8×10^4 cpm of nick-translated pDmh 604 DNA, approx. 7 μ g E. coli 4S carrier DNA, 4 x SSC and 50% formamide, covered each preparation. The slides were incubated at 37°C for 14-18 $\frac{1}{2}$ hrs.

e) Processing of ^3H -18+28S rRNA In Situ Hybridised Slides For Autoradiography

All preparations were rinsed in 2 x SSC several times after incubation in ^3H -18+28S rRNA. Salivary gland squash preparations were then subjected to an RNAase treatment (from bovine pancreas, Boehringer Mannheim GmbH) at a concentration of 20 μ g/ml at 37°C for 60 min., in order to remove unspecifically bound ^3H -18+28S rRNA from the slide. Alternatively, larval brain ganglia squash preparations were not treated with RNAase, in order to avoid digestion of any ^3H -18+28S rRNA specifically bound to nucleolus organizing regions in the diploid nuclei. After ribonuclease treatment, the slides were rinsed several times in 2 x SSC for 5 min. each, followed by an ice cold 5% TCA treatment in 2 x SSC for 5 min. After an intensive wash of the slides in 2 x SSC, they were transferred to 70% and 96% ethanol and finally air-dried.

f) Processing of pDmh 604 DNA-Hybridised Slides For Autoradiography

The slides were treated as recommended by Hennen et al. (1975) without any DNAase treatment for removal of unspecifically bound pDmh 604 DNA from the slides. The further processing of the preparations for autoradiography corresponds with the one mentioned in section II.2.7.e).

g) Autoradiography and Staining of Cytological Preparations

All slides were covered with Kodak-AR10 stripping film and exposed at 4°C. Salivary gland squash preparations, hybridised with ^3H -18+28S rRNA or nick-translated pDmh 604 DNA, were exposed for 7-14 days. Larval brain ganglia squash preparations, hybridised with ^3H -18+28S rRNA, were left to expose for 135-140 days.

The slides were developed in Kodak D-19 developer at 15°C for 5 min., fixed, and rinsed under running tap water for at least 20 min.

The dry slides were stained in a freshly prepared, buffered 0.25% toluidine blue O (Merck) solution at pH 3.9. The buffer consisted of citric acid (10.88 g/l), 0.1N NaOH and 0.05N HCl.

II.2.8. Silver-Staining of Polytene and Mitotic Chromosomes

Polytene and mitotic chromosomes of D. affinis and D. subobscura as well as mitotic chromosomes of D. melanogaster were prepared as outlined in section II.2.2. and II.2.3. The preparations were treated according to the AgAS-technique of Goodpasture and Bloom (1975): the slides were first rinsed in bidistilled water and then flooded with 50% AgNO_3 in bidistilled water (w/v). A cover-slip was placed upon the AgNO_3 -solution on the slide. Cytological preparations of salivary glands were then

placed under a 75W-lamp at a distance of 10 cm for 20 min. After cooling of the slides at room temperature for 5-10 min., the 50% AgNO_3 -solution was washed off the preparation with bidistilled water, and the slides were air-dried. Preparations of larval brain ganglia, alternatively, were transferred into a moist Petri-dish and were left for 24-36 hrs. at 40°C , followed by a thorough rinse in bidistilled water.

To develop the slides following incubation in 50% AgNO_3 , two solutions were used. Solution A consisted of 4gr AgNO_3 in 10 ml 50% NH_4OH (pH 12), solution B was composed of 3.2 gr Na acetate in 100 ml 1% formaldehyde (pH 5). Optimal differentiation of silver stained polytene and mitotic chromosomes was obtained, when the solutions were aged for 2-3 days at 4°C prior to use. One drop of each solution was placed simultaneously aside the squashed cells on the slide. The two drops were mixed by first agitating and then covering the preparation with a cover-slip. The slide was immediately microscopically checked, and when the chromosomes were optimally differentiated, the slide was rinsed with distilled water and air-dried. Finally, the slides were mounted in Euparal.

II.2.9. Giemsa C-Staining of Mitotic Chromosomes

Cytological preparations of mitotic chromosomes from D. affinis, D. subobscura and D. melanogaster (see section II.2.3.) were treated with saturated $\text{Ba}(\text{OH})_2$ for 4-6 min., followed by a rinse under running tap water for approx. 20 min. and subsequent air-drying. They were then incubated at 67°C for 60 min. in 2 x SSC, shortly rinsed with distilled water, and stained in a buffered 2% Giemsa-solution (pH 7). Sorensen

buffer was used, consisting of 2 parts KH_2PO_4 (9.08 gr/l) and 3 parts Na_2HPO_4 (9.47 gr/l). The slides were stained for approx. 30 min. and then air-dried. The differentiation of the chromosomes could be improved, when the dry slides were briefly incubated in 96% ethanol prior to sealing in Euparal.

III. RESULTS

III.1. Analysis of the Polytene Karyotype of *Drosophila affinis*

The polytene karyotype of *Drosophila affinis* consists of five chromosomes. With reference to previously published designations of salivary gland chromosomes in the *D. affinis* subgroup (Miller, 1977), they are marked in the present work with capital letters. B, E and the X-chromosome are represented by two arms each; C and F consist of one arm each (Stone, 1962). Chromosome F is the shortest chromosome within the polytene complement, and presumably corresponds with the mitotic "dot"-chromosome of the same species.

As in other *Drosophila* species, the homologs of all autosomes - and in females all X-chromosomes - are intimately paired. All chromosomes are fused in their centromeric regions to form a prominent chromocenter, which consists of β -heterochromatin exclusively, i.e. the heterochromatin appears as diffusely condensed, granular chromatin throughout the chromocenter (Fig. 1), thus corresponding with the definition of β -heterochromatin according to Heitz (1934). In male polytene karyotypes, the Y-chromosome cannot be detected by the preparation techniques applied in this work and, therefore, is unlikely to be po-

lytenized in the course of larval development.

The bands on the chromosomes are cytologically mapped and divided into 207 regions (arabic numbers), as is shown in Fig 2. Each region is further divided into two subdivisions (capital letters), to each of which a number of bands is attributed that are designated in the text with small letters. The total number of bands in the polytene chromosome complement amounts to approx. 1200 but most likely represents an underestimate of the absolute band number, as the resolution of the light microscope does not permit optical separation of closely adjacent bands in any case. The centromere near (proximal) ends of the euchromatic chromosome arms coincide with the start points in measuring chromosome arms. Correspondingly, the position number of chromosomal regions increases from the proximal to the distal end in any given chromosome arm.

III.1.1. Chromosome B

a) Short Arm

For cytological band mapping on the short arm of chromosome B, the relative positions of the 22 sites listed in Tab. 1a) have been determined. The location of sites with a relative position ≤ 0.1 or ≥ 0.9 respectively, was additionally referred to the distance (in units) between regions 5Ba and 15Bb (L') by the relation $\frac{l}{L}$, (l: distance between the relevant site and the proximal end of the chromosome arm) (Tab. 1b). The same calculation was performed for the corresponding sites with relative positions of ≤ 0.1 and ≥ 0.9 on the chromosome arms outlined in the following sections.

The data presented for the short arm of chromosome B (Tab. 1a) have been obtained from 6 individuals of 4 different stocks. The relative positions of those bands that are located between the sites listed in Tab. 1a) have been calculated by measuring their relative distance from their nearest proximal quantitatively registered site and, accordingly, have been marked in the chromosome map (Fig. 2).

Regions 2Aa-2Ab, and 6Ac-6Bb respectively, each exhibit two variants of banding patterns. 2Aa-2Ab either appears as two thin dotted bands (Fig. 3a) or as a condensed darkly stained double band (Fig. 3b). Both variants can be observed within the same salivary gland. Region 6Ac-6Bb is often observed as three condensed equidistant bands (Fig. 4a). Alternatively, the relevant region is represented as a discrete dotted band in 6Ac followed by a condensed double band in 6Bb (Fig. 4b).

In the developmental stages examined in this work, a consistent puffing pattern was observed, composed of a small puff each in region 1Aa-1Ba and 2Ab-2Ba and a medium sized puff in region 15Aa-15Bc. Examples for small, medium-sized and large puffs are illustrated in Fig. 16. The criteria on which the classification in puff-size is based are outlined in the legend to Fig. 16.

A picture of the total short arm of chromosome B is shown in Fig. 5.

b) Long Arm

The relative positions of 17 sites are listed in Tab. 2a). The location of sites at relative positions ≥ 0.9 is referred to the distance between 34Aa and 47Ab in the manner described in III.1.1.a). According to the approach outlined in the same section, bands, that are not listed in Tab. 2, were registered in the chromosome map (Fig. 2). The data pre-

sented in Tab. 2 derive from 3 individuals out of 2 different stocks. One of the individuals was homozygous for the inverted part between regions 33Aa and 37Ba. Another individual was homozygous for an inverted segment between 30Aa and 37Ba. In measuring the distance between the listed sites in Tab. 2a) within the inverted parts and the proximal end of the chromosome arm, units were counted up to the proximal breakage site of the inversion, and subsequently counting was proceeded in reverse direction from the distal breakage site. Thus, data were obtained that are comparable to those from the standard gene arrangement.

The puffing pattern in the developmental stages observed consists of a small puff in region 29Ba-29Bb and medium-sized puffs in regions 25Ba-26Ac, 30Ba-31Ab, 34B, 36Aa and 39Aa-39Ba.

The total long arm of chromosome B is shown in Fig. 6.

III.1.2. Chromosome C

The relative positions of 18 sites have been calculated (Tab. 3a). The location of sites at relative positions ≤ 0.1 or ≥ 0.9 was referred to the distance between 62Aa and 77Bb (Tab. 3b). The data were obtained from 4 examined individuals out of 2 stocks.

The puffing pattern observed throughout the individuals studied consists of one medium-sized puff in region 68Ba-69Aa.

An illustration of chromosome C is shown in Fig. 7.

III.1.3. Chromosome E

a) Short Arm

The data on the relative positions of 12 sites are listed in Tab. 4a). The location of 103Bb at the relative position of 0.92 was additionally referred to the length of the segment between region 95Ac and 103Ab. 8 individuals from 3 stocks were used.

The observed puffing pattern consists of a medium-sized puff each in region 90Bb-91Aa and 91Ba-92Ab.

Region 101Ba-101Bd appears in two modifications within and between individuals. Fig. 8a shows the banding pattern in this region as it is designed in the chromosome map in Fig. 2: a proximal thin band (101Ba) is followed by a discrete dotted band and a more prominent condensed single band in region 101Bc. Alternatively, a dotted band appears in region 101Ba which is followed by a double band in region 101Bb and 101Bc (Fig. 8b).

The total short arm of chromosome E is shown in Fig. 9.

b) Long Arm

The relative positions of 18 sites were determined (Tab. 5a). The location of sites that are at relative positions of ≤ 0.1 or ≥ 0.9 are referred to the length of the segment between 113Bd and 125Ab (Tab.5b). 6 individuals out of 3 stocks were examined.

Region 106Aa-106Bc is occasionally included in the heterochromatic chromocenter (Fig. 10a) while remaining euchromatic in other nuclei from the same salivary gland (Fig. 10b).

A large puff consistently appears in region 112A-112B, whereas region 127Ba-128Ad presents a small puff.

The long arm of chromosome E is shown in Fig. 11.

III.1.4. Chromosome F

The relative positions of the proximal and the distal end of region 133Aa have been calculated and listed in Tab. 6. The data derive from 5 individuals out of 2 stocks.

Chromosome F is shown in Fig. 12.

III.1.5. Chromosome X

a) Short Arm

The short arm of chromosome X exhibits an intercalary heterochromatic block in region 135B, which occasionally appears fused with the chromocenter. Therefore, chromosomal segments in the vicinity of region 135B often become torn in cytological preparations, which makes it difficult to obtain intact arms suitable for complete analysis of their banding pattern.

In Tab. 7a) the relative positions of 10 sites between regions 136Aa and 160Bb are listed. In Tab. 7b) the relative length between region 134Aa and 135Ad is expressed in relation to the distance between region 136Aa and 160Bb. The location of sites at relative positions ≤ 0.1 or ≥ 0.9 is referred to the distance between 149Ab and 155Ad (Tab. 8). The data were obtained from a male and a female individual from two different stocks.

A small puff in region 147Aa-147Bb was consistently observed.

The banding pattern of the short arm of chromosome X is shown in Fig. 13.

b) Long Arm

The relative positions of 16 sites have been calculated (Tab. 9a). The location of sites at relative positions ≥ 0.9 was referred to the length between region 174Aa and 196Bc (Tab. 9b). The data were obtained from two females and 1 male from 2 stocks.

The banding pattern in region 196Aa-196Bc exhibits two modifications: either it is represented as a distinct double band in 196A followed by two double bands in 196B (Fig. 14a), or a thin condensed band in 196A is followed by two discrete dotted bands (196Bb and 196Bc) (Fig. 14b), such as it is designed in the chromosome map.

Region 176Aa-176Ab exhibits a medium-sized puff, and a large puff appears in region 206Aa.

The long arm of chromosome X is shown in Fig. 15.

Tab. 1a

Ar.	Total length (units)	2Aa	2Ab	3Aa	3Ab	5Aa	5Ba	6Aa	6Ac	7Aa	7Ab
1	133	10 0.07	11 0.08	17.5 0.13	19 0.14	27 0.20	31 0.23	34 0.25	37 0.28	42 0.31	44 0.32
2	142	9 0.06	10 0.07	20 0.14	22 0.15	31 0.22	32.5 0.23	38 0.27	42 0.30	46 0.32	48 0.34
3	110.5	9.5 0.09	11.5 0.10	14.5 0.13	17.5 0.16	24 0.22	28 0.25	30 0.27	34 0.31	38 0.34	39 0.35
4	117.5	9.5 0.08	11 0.09	15 0.13	18 0.15	25 0.22	29 0.25	31.5 0.27	34 0.31	39.5 0.34	41.5 0.35
5	116	9.5 0.08	12 0.10	15 0.13	19 0.16	27.5 0.24	31.5 0.27	33 0.28	36 0.31	40 0.34	42.5 0.37
6	137	10 0.07	13 0.09	22 0.16	23 0.17	31 0.23	34 0.25	37.5 0.27	40 0.29	46 0.34	49.5 0.36
7	104	8 0.08	9 0.09	14 0.13	15 0.14	20 0.19	22 0.21	25 0.24	28 0.27	31 0.30	33.5 0.32
8	125.5	9 0.07	10.5 0.08	18 0.14	20 0.16	29 0.23	31 0.25	35 0.28	37 0.29	40.5 0.32	42 0.33
9	109	7 0.06	8 0.07	14 0.13	16 0.15	22 0.20	25 0.23	27 0.25	31 0.28	34 0.31	37 0.34
10	112	9.5 0.08	11 0.10	21 0.18	22 0.20	29.5 0.26	33.5 0.30	36 0.32	39.5 0.35	42.5 0.38	44.5 0.40
$\bar{x} \pm s$	120.7 ± 10.9	0.075 ± 0.01	0.087 ± 0.01	0.14 ± 0.01	0.158 ± 0.02	0.221 ± 0.02	0.247 ± 0.02	0.27 ± 0.02	0.297 ± 0.02	0.33 ± 0.02	0.348 ± 0.02

Tab. 1a: Relative positions of sites on the short arm of chromosome B. In each line, data obtained from one chromosome arm are listed. The upper numbers refer to the measured distance between a defined site and the proximal end of the chromosome arm. The lower numbers express the position of this site relative to the total length of the chromosome arm. In the first chromosome arm listed, regions 15Aa and 15Bb could not be identified.

Tab. 1a (cont.)

8Aa	10Aa	11Ba	13Aa	14Aa	14Bb	15Aa	15Bb	18Aa	19Bc	21Ab	21Ba
57 0.43	65 0.49	73 0.55	79 0.59	81 0.61	87 0.65	?	?	112 0.84	120 0.90	127 0.95	129 0.97
56 0.39	62.5 0.44	68 0.48	78 0.55	84 0.59	88 0.62	89 0.63	90 0.63	100 0.70	109 0.76	117.5 0.83	119 0.84
44 0.40	49.5 0.45	55 0.50	64 0.58	69 0.62	76 0.66	77 0.70	81 0.73	87 0.79	100.5 0.91	103.5 0.94	106.5 0.96
47.5 0.40	55 0.47	56 0.48	67 0.57	72 0.61	77 0.66	77.5 0.66	80.5 0.69	93 0.79	102.5 0.87	110 0.94	113 0.96
49.5 0.43	57.5 0.50	63 0.54	72.5 0.63	76 0.66	80 0.69	82 0.71	82.5 0.71	89 0.77	97.5 0.84	103 0.89	105.5 0.91
59 0.43	68.5 0.50	78 0.57	94.5 0.69	98.5 0.72	105 0.77	106 0.77	108 0.79	118.5 0.86	126 0.92	132 0.96	134 0.98
40 0.38	48.5 0.47	61 0.59	64 0.62	68 0.65	70 0.67	72.5 0.68	74 0.71	83.5 0.80	90.5 0.87	97 0.93	99 0.95
45 0.36	56.5 0.45	59.5 0.47	72 0.57	74 0.59	86 0.69	92 0.73	95 0.76	104 0.83	113.5 0.90	120 0.96	122 0.97
46 0.41	50.5 0.46	55 0.53	68 0.62	72 0.66	77 0.71	78.5 0.72	80 0.73	91 0.83	98 0.90	102 0.94	104 0.95
50 0.45	55.5 0.50	60 0.54	73 0.65	77 0.69	79.5 0.71	80 0.71	82 0.73	90 0.80	98 0.88	105 0.94	107 0.95
0.408 +0.03	0.473 +0.02	0.525 +0.04	0.607 +0.04	0.640 +0.05	0.685 +0.04	0.700 +0.04	0.720 +0.04	0.801 +0.05	0.875 +0.04	0.928 +0.04	0.944 +0.04

Tab. 1b:

Nr.	5Ba-18Aa (units)	2Aa	2Ab	21Ab	21Ba
1	81	10 0.12	11 0.14	127 1.57	129 1.59
2	67.5	9 0.13	10 0.15	117.5 1.74	119 1.76
3	59	9.5 0.16	11.5 0.20	100.5 1.70	106.5 1.81
4	64	9.5 0.17	11 0.21	110 1.72	113 1.77
5	57.5	9.5 0.17	12 0.21	103 1.79	105.5 1.83
6	84.5	10 0.12	13 0.15	132 1.56	134 1.59
7	61.5	8 0.13	9 0.15	97 1.58	99 1.61
8	73	9 0.12	10.5 0.14	120 1.64	122 1.67
9	66	7 0.11	8 0.12	102 1.55	104 1.58
10	56.5	9.5 0.17	11 0.19	105 1.86	107 1.90
$\bar{x} \pm s$	67.05 ± 9.78	0.138 ± 0.06	0.162 ± 0.03	1.671 ± 0.12	1.711 ± 0.18

Tab. 1b: The position of sites relative to the distance between region 5Ba and 18Aa (lower numbers). The upper numbers refer to the distance between a defined site and the proximal end of the chromosome arm.

Tab. 2a:

Nr.	Total length (units)	27Ab	29Ab	32Aa	32Bc	34Aa	35Ab	36Ba	37Ba
1	180	25 0.14	39 0.22	56 0.31	66.5 0.37	72.5 0.40	82 0.46	102 0.54	114 0.60
2	204	35 0.17	49 0.24	67 0.33	76 0.37	84 0.41	94 0.46	103 0.50	114 0.56
3	196	30 0.15	42 0.21	60 0.31	68 0.35	74 0.38	84 0.43	93 0.47	101 0.52
4	208	36 0.17	51.5 0.28	71 0.34	79 0.38	85 0.41	93 0.45	102 0.49	114 0.55
5	191	25 0.13	39 0.20	56 0.29	65 0.34	72 0.38	81 0.42	90 0.47	104 0.54
$\bar{x} \pm s$	195.8 ± 11.05	0.152 ± 0.02	0.230 ± 0.03	0.316 ± 0.02	0.362 ± 0.02	0.396 ± 0.02	0.444 ± 0.02	0.494 ± 0.03	0.554 ± 0.03

Tab. 2a (cont.):

Nr.	40Ba	41Ba	42Ba	43Ba	45Ba	46Ab	47Ab	48Aa prox.	48Aa dist.
1	124 0.69	131 0.73	138 0.77	146 0.81	157 0.87	162 0.90	166.5 0.93	175 0.97	177 0.98
2	136 0.67	141 0.69	148 0.73	159 0.78	175 0.86	184 0.90	189 0.93	198 0.97	199 0.98
3	120 0.61	129 0.66	135 0.69	149 0.76	166 0.85	175 0.89	183 0.93	191 0.97	192 0.98
4	141 0.68	147 0.71	157 0.75	167 0.80	180 0.87	187 0.90	194 0.93	202 0.97	204 0.98
5	119 0.62	126 0.66	134 0.70	144 0.75	157.5 0.82	167 0.87	178 0.93	185 0.97	187 0.98
$\bar{x} \pm s$	0.654 ± 0.04	0.690 ± 0.03	0.728 ± 0.03	0.780 ± 0.03	0.854 ± 0.02	0.892 ± 0.01	0.930	0.970	0.980

Tab. 2a: Relative positions of sites on the long arm of chromosome B.

For further explanations see text to Tab. 1a.

Chromosome arms Nr. 2, 3 and 4 originate from an individual carrying an inversion between region 33Aa and 37Ba. Chromosome arm Nr. 5 was obtained from an individual homozygous for an inversion between region 30Aa and 37Ba.

Tab. 2b:

Nr.	34Aa-47Ab (units)	47Ab	48Aa prox.	48Aa dist.
1	110.5	166.5 1.51	175 1.58	177 1.60
2	105	189 1.80	198 1.89	199 1.90
3	109	183 1.67	191 1.75	192 1.76
4	109	194 1.78	202 1.85	204 1.87
5	106	178 1.68	185 1.75	187 1.76
$\bar{x} \pm s$	107.9 ± 2.3	1.69 ± 0.12	1.76 ± 0.12	1.78 ± 0.12

Tab. 2b: The position of sites relative to the distance between region 34Aa and 47Ab (lower numbers). The upper numbers represent the distance (units) between the relevant site and the proximal end of the chromosome arm.

Tab. 3a:

Nr.	Total length (units)	51Ab	52Ba	54Aa	58Ab	62Aa	64Aa	66Aa	68Bd
1	292	15.5 0.03	22.5 0.08	45.5 0.16	73 0.25	100 0.34	121 0.41	142 0.49	161 0.55
2	250	12 0.05	19 0.08	42 0.17	67 0.27	92 0.37	109 0.44	124 0.50	137.5 0.55
3	285	15 0.06	22 0.08	42.5 0.15	66 0.23	90.5 0.32	114 0.40	129 0.45	150.5 0.53
4	230	13 0.06	20 0.09	37.5 0.16	61.5 0.27	77 0.34	89.5 0.39	103 0.45	117 0.51
5	270	12 0.04	18 0.07	38 0.14	65 0.24	90 0.33	108 0.40	125 0.46	143 0.53
6	248.5	13 0.05	15 0.06	38 0.15	66 0.27	91 0.37	102 0.41	116 0.47	127 0.51
7	314.5	14 0.05	21.5 0.07	51 0.16	81.5 0.26	112.5 0.36	130 0.41	149 0.47	167 0.53
$\bar{x} \pm s$	270 ± 29.30	0.055 ± 0.01	0.073 ± 0.01	0.156 ± 0.01	0.253 ± 0.01	0.345 ± 0.02	0.409 ± 0.02	0.470 ± 0.02	0.530 ± 0.02

Tab. 3a (cont.):

Nr.	69Bb	70Bb	73Ba	74Aa	75Aa	75Ab	77Bb	79Aa	81Ab	82Ab
1	171 0.59	185 0.63	209.5 0.72	214.5 0.74	224 0.77	226.5 0.78	241 0.83	247 0.85	269 0.92	277 0.95
2	144 0.58	155.5 0.62	170 0.68	181.5 0.73	188 0.75	189 0.76	205 0.82	210 0.84	230 0.92	238 0.95
3	162 0.57	175 0.61	196.5 0.69	206 0.72	212 0.74	214.5 0.75	234 0.82	240 0.84	262 0.92	271.5 0.95
4	123 0.54	133 0.58	149 0.65	157 0.68	165 0.72	167.5 0.73	183 0.80	188 0.82	210 0.91	217 0.94
5	153.5 0.57	166.5 0.62	185 0.69	195 0.72	202 0.75	204 0.76	222 0.82	228 0.84	246 0.91	255.5 0.95
6	133 0.54	142.4 0.57	159 0.64	167 0.67	176 0.71	178.5 0.72	196.5 0.79	203 0.82	224 0.90	233.5 0.94
7	176 0.56	191 0.61	208.5 0.66	221 0.70	229 0.73	230 0.73	257 0.82	264 0.84	289 0.92	299 0.95
$\bar{x} \pm s$	0.561 ± 0.03	0.606 ± 0.02	0.675 ± 0.03	0.709 ± 0.02	0.737 ± 0.02	0.745 ± 0.02	0.813 ± 0.01	0.835 ± 0.01	0.915 ± 0.01	0.948 ± 0.01

Tab. 3a: Relative positions of sites on chromosome C. For further explanations see text to Tab. 1a.

Tab. 3b:

Nr.	62Aa-77Bb (units)	51Ab	52Ba	81Ab	82Ab
1	141	15.5 0.11	22.5 0.16	269 1.91	277 1.96
2	123	12 0.10	19 0.15	230 1.87	238 1.93
3	143.5	15 0.10	22 0.15	262 1.83	271.5 1.89
4	106	13 0.12	20 0.19	210 1.98	217 2.05
5	132	12 0.09	18 0.14	246 1.86	255.5 1.94
6	105.5	13 0.12	15 0.14	224 2.12	233.5 2.21
7	144.5	14 0.10	21.5 0.15	289 2.00	299 2.07
$\bar{x} \pm s$	127.92 ± 16.90	0.106 ± 0.01	0.154 ± 0.02	1.939 ± 0.10	2.007 ± 0.11

Tab. 3b: Positions of sites relative to the distance (units) between region 62Aa and 77Bb (lower numbers). The upper numbers represent the distance between the defined site and the proximal end of the chromosome arm.

Tab. 4a:

Nr.	Total length (units)	88Ba	90Aa	91Ab	92Ba	93Bc	94Ad	95Ac	95Bd
1	157	18.5 0.12	29.5 0.19	49 0.31	59 0.38	57 0.48	80.5 0.51	92 0.59	97 0.62
2	164	23 0.14	32.5 0.20	47 0.29	63 0.38	73 0.45	77.5 0.47	80 0.49	87 0.53
3	164.5	29 0.18	38 0.23	49 0.30	61.5 0.37	74 0.42	80 0.47	83 0.50	89 0.55
4	152.5	21.5 0.14	31.5 0.21	45 0.30	56 0.35	64 0.43	71 0.46	76 0.50	84 0.55
5	149	20 0.13	29 0.19	44.5 0.30	52 0.35	63.5 0.43	69 0.46	74.5 0.50	82 0.55
6	126	18 0.14	30 0.24	37 0.29	47 0.37	50 0.40	55 0.44	60 0.48	65 0.52
7	129.5	17 0.13	26 0.20	35 0.27	43.5 0.34	52 0.40	58 0.45	60 0.46	64 0.49
8	137	16 0.12	24.5 0.18	35 0.26	48.5 0.35	59 0.43	63 0.46	68 0.50	73 0.53
$\bar{x} \pm s$	147.44 ± 15.00	0.138 ± 0.03	0.182 ± 0.03	0.257 ± 0.04	0.320 ± 0.05	0.431 ± 0.03	0.468 ± 0.02	0.502 ± 0.04	0.542 ± 0.04

Tab. 4a: Relative positions of sites on the short arm of chromosome E.

For further explanations see text to Tab. 1a.

Tab. 4a (cont.):

Nr.	99Bb	102Ac	103Ab	103Bb
1	119 0.76	128 0.82	136 0.87	146 0.93
2	118 0.72	131 0.80	142 0.82	150 0.92
3	124.5 0.75	133 0.81	143 0.87	149 0.91
4	113 0.74	123 0.81	133 0.87	139.5 0.91
5	111 0.75	129 0.83	132 0.89	136 0.91
6	91 0.72	102 0.80	109 0.87	115 0.91
7	94.5 0.73	105 0.81	112 0.87	118 0.91
8	101.5 0.74	112 0.82	119.5 0.87	125 0.91
$\bar{x} \pm s$	0.737 ± 0.01	0.812 ± 0.01	0.871 ± 0.01	0.916 ± 0.01

Tab. 4b:

Nr.	95Ac-103Ab (units)	103Bb
1	44	146 3.32
2	62	150 2.42
3	60	149 2.48
4	57	139.5 2.45
5	57.5	136 2.37
6	49	115 2.35
7	52	118 2.26
8	51.5	125 2.43
$\bar{x} \pm s$	56.63 ± 8.63	2.51 ± 0.33

Tab. 4b: Positions of sites relative to the distance between region 95Ac and 103Ab (lower numbers). The upper numbers refer to the distance between the defined site and the proximal end of the chromosome arm.

Tab. 5a:

Nr.	Total length (units)	107Be	109Ba	110Aa	111Bc	113Ac	113Bd	115Ba
1	203	15 0.07	36 0.18	37 0.18	50 0.25	59 0.29	71.5 0.35	80 0.39
2	219	14.5 0.07	39 0.18	41 0.19	52.5 0.24	66 0.30	76.5 0.35	90 0.41
3	206	19.5 0.10	36 0.18	37 0.18	48 0.23	64 0.31	70 0.34	82 0.40
4	204	17 0.08	35 0.17	36 0.18	44 0.22	57 0.28	69 0.34	77 0.38
$\bar{x} \pm s$	208 ± 7.44	0.08 ± 0.01	0.178 ± 0.002	0.183 ± 0.005	0.235 ± 0.01	0.295 ± 0.01	0.345 ± 0.01	0.395 ± 0.01

Tab. 5a (cont.):

Nr.	117Ba	118Aa	121Ab	123Bb	125Ab	127A prox.	127A dist.	129Bb prox.
1	95 0.47	103 0.51	127 0.63	143 0.70	156 0.77	168 0.83	172 0.85	187.5 0.92
2	107 0.49	113 0.52	141 0.64	158.5 0.72	170 0.78	182 0.83	184 0.84	200.5 0.92
3	87 0.42	95 0.46	121 0.59	136 0.66	152 0.74	164 0.80	166 0.81	190 0.92
4	92 0.45	100 0.49	125 0.61	143 0.70	154 0.76	166 0.81	167.5 0.82	187 0.92
$\bar{x} \pm s$	0.458 ± 0.03	0.495 ± 0.03	0.618 ± 0.02	0.695 ± 0.03	0.763 ± 0.02	0.818 ± 0.02	0.830 ± 0.02	0.920

Tab. 5a: Relative positions of sites on the long arm of chromosome E.

For further explanation see text to Tab. 1a.

Tab. 5a (cont.):

Nr.	129Bb dist.	130Bb	130Bd
1	189 0.93	195 0.96	197 0.97
2	202 0.92	209 0.95	211.5 0.97
3	192 0.93	200 0.97	202 0.98
4	189 0.93	195.5 0.96	197 0.97
$\bar{x} \pm s$	0.928 ± 0.005	0.960 ± 0.01	0.972 ± 0.005

Tab. 5b:

Nr.	113Bd-125Ab (units)	107Be	129Bb prox.	129Bb dist.	130Bb	130Bd
1	84.5	15 0.18	187.5 2.21	189 2.24	195 2.31	197 2.33
2	93.5	14.5 0.16	200.5 2.14	202 2.16	209 2.24	211.5 2.26
3	82	19.5 0.24	190 2.32	192 2.34	200 2.44	202 2.46
4	85	17 0.20	187 2.20	189 2.22	195.5 2.30	197 2.31
$\bar{x} \pm s$	86.25 ± 5.01	0.195 ± 0.03	2.218 ± 0.08	2.240 ± 0.07	2.323 ± 0.08	2.340 ± 0.09

Tab. 5b: Positions of sites relative to the distance between region 113Bd and 125Ab (lower numbers). The upper numbers represent the distance between a defined site and the proximal end of the chromosome arm.

Tab. 6:

Nr.	Total length (units)	133Aa prox.	133Aa dist.
1	15.5	8.5 0.55	10 0.65
2	17	8.5 0.50	10.5 0.62
3	14	7 0.50	8.5 0.61
4	13.5	6 0.44	7.5 0.56
5	15	8 0.53	10.5 0.70
$\bar{x} \pm s$	15 ± 1.34	0.504 ± 0.04	0.628 ± 0.07

Tab. 6: Relative positions of sites on chromosome F.
For further explanations see text to Tab. 1a.

Tab. 7a:

Nr.	136A-159B (units)	137Ba	138Bc	140Ab	143Ba	148Ab	148Bd	150Aa
1	200	12.5 0.06	26 0.13	43 0.22	71 0.36	94 0.47	108 0.54	118 0.59
2	213.5	7 0.03	16 0.08	33 0.16	58 0.27	86 0.40	108 0.51	118 0.55
3	193	14 0.07	21 0.11	?	?	70 0.36	89 0.46	99 0.51
$\bar{x} \pm s$	202.17 ± 10.42	0.050 ± 0.02	0.106 ± 0.02	0.190 ± 0.04	0.315 ± 0.06	0.410 ± 0.05	0.503 ± 0.04	0.550 ± 0.04

Tab. 7a (cont.):

Nr.	154Ad	158Aa	159Ba
1	153 0.77	179 0.90	189 0.95
2	158 0.74	188 0.88	200 0.94
3	134 0.69	165.5 0.86	180 0.93
$\bar{x} \pm s$	0.733 ± 0.04	0.880 ± 0.02	0.940 ± 0.01

Tab. 7b:

Nr.	134Aa-135Ad (units)	136Aa-159Bb (units)
1	14 0.07	200
2	20 0.09	213.5
$\bar{x} \pm s$	0.08 ± 0.01	

Tab. 7a: Relative positions of sites on the short arm of chromosome X.

The sites distal to the heterochromatic region 135B have been located, referring to the distance between region 136A and 159B (lower numbers). The upper numbers refer to the distance between a defined site and region 136Aa. In chromosome arm Nr. 3, regions 140Ab and 143Ba could not be identified.

Tab. 7b: Relative distance between regions 134Aa and 135Ad as referred to the distance between regions 136Aa and 159Bb.

Tab. 8:

Nr.	148Ab-154Ad (units)	159Ad	137Ba	138Bc
1	60	189 3.15	12.5 0.21	26 0.43
2	72	200 2.78	7 0.10	16 0.22
3	64	180 2.81	14 0.22	21 0.33
$\bar{x} \pm s$	65.33 ± 6.11	2.913 ± 0.21	0.177 0.07	0.327 0.11

Tab. 9: Position of sites distal to region 135B relative to the distance between region 148Ab and 154Ad (lower numbers). The upper numbers refer to the distance between the relevant site and region 136Aa.

Tab. 9a:

Nr.	Total length (units)	166Aa	167Aa	170Bb	174Aa	179Aa	183Ac	185Ac
1	367	43 0.12	50 0.14	85 0.23	107 0.29	153 0.42	179 0.49	206.5 0.56
2	295	29 0.10	36.5 0.12	60 0.20	82 0.28	122.5 0.42	142 0.48	162 0.55
3	285	42 0.15	49 0.17	76 0.27	94 0.33	132 0.46	146 0.51	157.5 0.53
$\bar{x} \pm s$	315.67 ± 44.74	0.121 ± 0.03	0.144 ± 0.02	0.234 ± 0.03	0.300 ± 0.03	0.432 ± 0.03	0.494 ± 0.02	0.548 ± 0.02

Tab. 9a (cont.):

Nr.	186Ba	188Ba	190Ac	195Ba	196Bc	199Bb	200Ab	205Ba	206Ab
1	212 0.58	223 0.61	239.5 0.65	274 0.75	286 0.78	303.5 0.83	310 0.85	343 0.94	354 0.97
2	167.5 0.57	180 0.61	190 0.64	214 0.73	224 0.76	235 0.80	238 0.81	278.5 0.94	285 0.97
3	160 0.56	168 0.59	174 0.61	198 0.70	203 0.71	? ?	? ?	265 0.93	274 0.96
$\bar{x} \pm s$	0.570 ± 0.01	0.603 ± 0.02	0.633 ± 0.02	0.727 ± 0.03	0.750 ± 0.03	0.815 ± 0.02	0.830 ± 0.03	0.936 ± 0.01	0.964 ± 0.003

Tab. 9a: Relative positions of sites on the long arm of chromosome X.

For further explanation see text to Tab. 1a. Regions 199Bb and 200Ab could not be identified on chromosome arm Nr. 3.

Tab. 9b:

Nr.	174Aa-196Bc (units)	205Ba	206Ab
1	179	343 1.92	354 1.98
2	142	278.5 1.96	285 2.01
3	109	265 2.43	274 2.51
$\bar{x} \pm s$	143.33 ± 35.02	2.10 ± 0.28	2.17 ± 0.28

Tab. 9b: Position of sites relative to the distance between region 174Aa and 196Bc (lower numbers). The upper numbers represent the distance between a defined site and the proximal end of the chromosome arm.

Tab. 10:

Chromosome	Region	Extend
B (short arm)	1Aa-1Ba	+
	2Ab-2Ba	+
	15Aa-15Bc	++
B (long arm)	25Ba-26Ac	++
	29Ba-29Bb	+
	30Ba-31Ab	++
	34B	++
	36Aa	++
	39Aa-39Ba	++
C	68Ba-69Aa	++
E (short arm)	90Bb-91Aa	++
	91Ba-92Ab	++
E (long arm)	112A-112B	+++
	127Ba-128Ad	+
F	-	
X (short arm)	147Aa-147Ab	+
X (long arm)	176Aa-176Ab	+
	207Aa	+++

Tab. 10: List of puffed regions in the polytene karyotype of D. affinis.

+ small puff, ++ medium sized puff, +++ large puff.

III.2. In Situ Hybridisation Experiments With ^3H -18+28S rRNA

III.2.1. In Situ Hybridisation to Polytene Nuclei of D. affinis and D. subobscura

In polytene nuclei from salivary glands of D. affinis, D. subobscura and D. melanogaster, that had been hybridised with ^3H -18+28S rRNA from an in vitro cultured Echaliér cell line from D. melanogaster, only the nucleoli exhibited autoradiographic label, whereas the polytene chromosomes remained unlabelled.

In the three species, autoradiographs were stained with toluidine blue, which preferentially stains a distinct area within the nucleolus while leaving the surrounding nucleolar material almost unstained. This is the reason, why nucleoli are not visible in total if stained in the outlined manner and observed in light field.

In D. subobscura, a minimum of 21 days exposure time was necessary to obtain significant autoradiographic label over nucleoli. A nucleolus was considered as significantly labelled if its toluidine blue darkly staining area was covered by at least 5 silver grains. The number of silver grains that was attributed to background was calculated as the mean grain number from 10 unit areas equally sized as the labelled area. This value was subtracted from the absolute grain count over the nucleolus. Slides on which DNA denaturation had been omitted appeared entirely unlabelled over nucleoli. In a hybridisation series which had been exposed for 21 days, 77 (93.9%) out of 82 examined nuclei showed label in the darkly toluidine blue stained nucleolar area and over a part of the unstained surrounding nucleolar material (Fig. 17), indicating the presence of rDNA. A slide left to expose for 45 days

exhibited the same labelling pattern in 45 (95.7%) out of 47 examined nuclei. The values corrected for background are shown in the diagrams in Fig. 18a and b. In the series exposed for 21 days, the mean label intensity amounted to 9.9 ± 3.8 silver grains per toluidine blue darkly staining area. The series left to expose for 45 days exhibited 10.8 ± 3.7 silver grains as mean label intensity over the corresponding area. Neither the mean label intensities in the two series examined, nor the percentage of labelled nuclei significantly differed from each other, indicating that saturated label intensity is reached after 21 days of exposure. The absence of increase in label intensity after 45 days of exposure may be regarded as the result of silver grain fading in the course of prolonged exposure time (Rogers, 1967).

Also in D. affinis, autoradiographic label expanded over the toluidine blue dark area and a part of the unstained material within the nucleolus (Fig. 19). 37 (94.9%) out of 39 nucleoli were labelled after 30 days exposure. Slides that were not subjected to any DNA denaturation treatment did not show any label. To determine the mean label intensity, nucleoli exhibiting a minimum label of at least 5 silver grains over their darkly staining area were selected for evaluation. A mean label intensity of 32.6 ± 13.9 silver grains per nucleolus was calculated after correcting for background in the same manner as described for D. subobscura.

In a preparation of D. melanogaster used as control slide, 14 (93.3%) out of 15 nucleoli were labelled over most of the nucleolus (Fig. 20) with a mean label intensity of 17.6 ± 7.7 silver grains after an exposure time of 21 days. The minimum label intensity was 5 silver grains over the toluidine blue dark area within the nucleolus.

The percentage of labelled nuclei does not significantly differ between the three species examined. The distribution of autoradiographic label over nucleoli must be interpreted as being due to the presence of rDNA predominantly in the darkly toluidine blue staining area and extending towards the nucleolar periphery, because the toluidine blue positive area appeared labelled in each labelled nucleolus, whereas the unstained part exhibited a variable amount of label within each species.

III.2.2. In Situ Hybridisation to Diploid Nuclei From Larval Brain Ganglia of *D. affinis* and *D. subobscura*

Squash preparations of male larval brain ganglia from *D. affinis*, *D. subobscura* and *D. melanogaster* were hybridised with ^3H -18+28S rRNA from an in vitro cultured Echalier cell line from *D. melanogaster*. All three species did not exhibit significant autoradiographic label over metaphase chromosomes after an exposure time of 135-140 days. Labelled interphases were observed in all three species. Diploid interphases were identified according to their small size as compared to the larger tetraploid interphases within the same tissue (Fig. 21).

The background level on many slides was too high to allow a quantitative analysis of the labelling patterns. Therefore, the data presented below have been obtained from those 1-2 preparations from each species examined which exhibited almost no background.

In D. affinis, 742 (58.5%) out of 1268 diploid nuclei of the same preparation were labelled. The extend of label in a random sample of 100 labelled nuclei was determined, resulting in a mean label intensity of 3.1 ± 1.3 silver grains per diploid interphase after correction for background by subtracting 1 silver grain from each grain count. The preparation had been exposed for 140 days. An interphase was regarded as significantly labelled if it was covered by at least 2 silver grains. A control slide, that had not been submitted to the DNA denaturation treatment prior to incubation in the hybridisation mixture, did not exhibit any significant label over interphases.

In some toluidine blue-stained diploid interphases, a more darkly stained area was observed, which was covered by a cluster of 5 or more silver grains (Fig. 22a). The significance of this observation will be pointed out in section III.3.2.

In D. subobscura, 542 (51.5%) out of 1052 diploid interphases on the same slide were labelled. Silver grains over a random sample of 100 nuclei per preparation were counted on two slides of different experimental series: mean label intensities of 3.4 ± 1.3 silver grains per diploid interphase on the first slide and 3.2 ± 1.4 silver grains on the second slide after an exposure time of 140 days in both series was calculated after correction for background in the manner outlined for D. affinis. The minimum label intensity limit was set at 2 silver grains per interphase. A control slide without DNA denaturation treatment prior to in situ hybridisation of the tritiated rRNA probe did not exhibit any significant label over interphases.

Similar to the observations made in D. affinis, some toluidine blue stained interphases exhibited a more darkly stained area covered with a cluster of silver grains (Fig. 22b).

In D. melanogaster, autoradiographic label was found over 575 (53.0%) out of 1085 diploid interphases. At a minimum silver grain number of 2 per diploid interphase in a random sample of 100 labelled nuclei, 3.2 ± 1.0 silver grains were calculated as mean label intensity. The slide had been exposed for 135 days.

The frequencies of label intensity within randomly chosen samples of 100 labelled nuclei each in D. affinis, D. subobscura and D. melanogaster are shown in Fig. 23a-c. The values presented in the diagrams are corrected for background. According to the z-test, the mean label intensities do not significantly differ between the species examined.

III.3. Silver-Staining of Polytene and Diploid Nuclei in D. affinis and D. subobscura

Silver-staining of chromatin by the AgAS-technique described by Goodpasture and Bloom (1975) on mammalian chromosomes, allows the detection of transcriptionally active nucleolus organizer regions (NORs). The method was, therefore, applied in the present work to see whether sites of ribosomal cistrons can be detected histochemically, after the in situ hybridisation experiments with ^3H -18S rRNA from D. melanogaster had failed to reveal the respective locations.

III.3.1. Polytene Nuclei

In both species, D. affinis and D. subobscura, the polytene chromosomes exhibit a differential, consistent staining pattern, i.e. throughout the karyotypes distinct positively Ag-staining bands appear dark-brown, as compared to the yellowish non-differentially staining regions. No darkly staining interbands have been observed. In D. affinis and D. subobscura, approximately 50 Ag-positive bands per karyotype in each species were observed. Because of the uniform yellowish appearance of the remaining regions, the general banding pattern of the polytene chromosomes appears indistinct, thus rendering the identification of Ag-labelled intercalary bands more difficult. To examine the consistency of the Ag-staining pattern, the location of positively Ag-staining bands was checked in a terminal segment of the X-chromosome in both species. In D. affinis, regions 202B and 206B at the distal end of the long arm of chromosome X exhibit Ag-positive staining. This pattern was observed in 19 nuclei from 2 preparations. The Ag-staining patterns of the long and the short arm of chromosome X are shown in Fig. 24a and b. Both arms together comprise approximately 17 darkly staining Ag-bands.

In D. subobscura, the Ag-staining pattern at the distal end of chromosome A (A=X) was studied. Positively Ag-staining bands were found in regions 15C, 16B and 16C. To identify these regions, the salivary gland chromosome map of Kunze-Mühl and Müller (1958) was used. This pattern was observed in 40 nuclei from 4 preparations. A total of approximately 8 positively Ag-staining bands on chromosome A was found (Fig. 25).

The observation on band-consistent Ag-staining patterns in salivary gland chromosomes might invoke the objection that in spite of the claimed Ag-specificity for actively transcribing NORs in mitotic chromosomes polytene darkly Ag-staining bands might reflect genetic activity at sites of other genes in addition to ribosomal cistrons. However, the observation that puffs as transcriptionally active sites mostly appear as non-differentially staining regions after application of the AgAS-technique (Fig. 26a and b), leads to the conclusion that Ag-positive bands represent regions of some other kind of specificity than attained in any genetically active region.

Nucleolar activity in both species is indicated by Ag-positive staining of the darkly toluidine blue staining area within the nucleolus (Fig. 27). While darkly silver-staining bands appear to be consistent within and between individuals, nucleoli often exhibit yellowish Ag-staining, presumably as a result of changes in genetic activity of nucleolar ribosomal cistrons, that might occur in the course of pupation.

Occasionally, association of nucleoli to chromosomal sites were observed in D. subobscura. However, in general, these sites did not coincide with darkly silver-staining bands, therefore are not likely to exhibit NOR-activity, and are supposed to be randomly associated to the nucleolus (Fig. 28).

III.3.2. Diploid Nuclei

In D. affinis and D. subobscura, metaphase chromosomes and interphases from larval brain ganglia stain differentially by the AgAS-technique. To examine the correlation between positively Ag-staining regions and the position of NORs on mitotic chromosomes, preparations of D. melanogaster larval brain ganglia were stained in the same manner and used as control preparations. In this species, NORs are known to be located adjacent to the proximal heterochromatin on the X-chromosome and on the short arm of the Y-chromosome (Ritossa and Spiegelmann, 1965).

In D. affinis ($2n = 10$), the male metaphase karyotype consists of 2 pairs of submetacentric autosomes, one pair of acrocentric autosomes, one pair of dot-like autosomes, as well as of a large metacentric X-chromosome and a J-shaped polymorphic Y-chromosome (Miller and Roy, 1964). Positively silver-staining sites appear on the acrocentric homologs in centromere-near position, on the dot-like chromosomes, near the centromere of the X-chromosome, and at the proximal end as well as in intercalary position of the Y-chromosome (Fig. 30b).

This staining pattern was observed in all metaphases examined on 4 preparations from 2 different series. Occasionally, the metacentric autosomes appeared slightly more darkly stained in centromere proximity. However, this pattern was less distinct as compared to the centromere-near positively Ag-stained sites on the acrocentric autosomes and on the X-chromosome; Moreover, these regions did not stain consistently throughout all metaphases examined.

Giemsa C-stained metaphases reveal heterochromatic blocks on all chromosome arms adjacent to the centromeres of all except the dot-like chromosomes (Fig. 30a); apparently, to some extent, heterochromatin tends to react positively to the AgAS-technique. However, the staining intensities allow a clear distinction between heterochromatin and the consistently positively dark-brown Ag-staining regions.

The male karyotype of D. subobscura ($2n=12$) consists of 4 pairs of acrocentric autosomes, one pair of dot-like chromosomes, one acrocentric X-chromosome and a small rod-shaped Y-chromosome.

The Giemsa C-banded karyotype exhibits heterochromatin in the centromeric regions of the 1st autosome pair and of the X-chromosome. The Y-chromosome usually appears entirely heterochromatic (Fig. 29a).

Ag-staining resulted in a similar banding pattern as Giemsa C-banding, revealing Ag-labelling on the 1st autosome pair and on the X-chromosome, both in centromere-near position, while producing Ag-positive staining over most of the Y-chromosome, yet leaving a short segment at its proximal end unstained.

The silver-staining pattern of D. subobscura metaphases is shown in Fig. 29b. This pattern was observed in all metaphases on 4 preparations from 2 series.

In the male metaphase karyotype of D. melanogaster (Fig. 31b), the X-chromosome exhibits specific silver-staining in the location of ribosomal cistrons in the proximal part of the X-chromosome, compared to the centromere-near regions of autosomes 2, 3 and 4, which also consist of heterochromatin as shown in their Giemsa C-banding pattern (Fig. 31a) and yet only occasionally stain by the AgAS-technique. The Y-chromosome shows Ag-positive staining on the short arm.

The results on Ag-stained metaphases make apparent that in the three species examined, positively Ag-stained sites with consistent dark-brown staining intensity can be distinguished from heterochromatic regions, that variably react positively to the AgAS-technique. The fact, that in D. melanogaster the consistently darkly Ag-staining sites occur in regions known to represent NORs (Ritossa and Spiegelmann, 1965), leads to the conclusion that the Ag-positive regions consistently found in D. affinis and D. subobscura metaphases also represent transcribed NORs.

The application of the AgAS-technique to polytene and mitotic nuclei shows that the relation between the pattern of consistently Ag-staining polytene sites and the location of NORs in metaphase karyotypes is rather complex, and that the nature of Ag-labelled polytene bands may be of other kind than originally presumed.

The differential Ag-staining pattern in diploid interphases yielded mainly two patterns in each species: in one pattern each nucleus showed 2 or more dark-brown areas (Fig. 32a-c), which occasionally seemed to adhere to form a staining pattern (Fig. 33a-c) equal in its appearance to the toluidine blue dark area within the interphase nucleus, which exhibits autoradiographic label after in situ hybridisation with ^3H -18+28S rRNA from *D. melanogaster* (Fig. 22a and b), thus, pointing to the presence of ribosomal cistrons in these Ag-positively staining areas.

For each species, 207-220 interphases from three individuals, including males and females, were examined with respect to the number of Ag-labelled areas per nucleus (Fig 34a-c). The mean number of these areas amounts to 3.85 ± 1.91 in D. affinis, 3.06 ± 1.56 in D. subobscura and 1.95 ± 0.96 in D. melanogaster. These data significantly differ from each other at the 0.1%-level as indicated by the F-test. The significant difference corresponds well with the species-specific number of transcriptionally active NORs found in metaphases, the latter amounting to 6-7 in D. affinis (6 in females, 7 in males), to 4 in D. subobscura and to 2 in D. melanogaster. Counts of more than 6-7 and 4 Ag-positive areas in D. affinis and D. subobscura, respectively of more than 2 in D. melanogaster, may be interpreted as being due to heterochromatic chromocenters, that also stain occasionally by the AgAS-technique in metaphases. Counts between 1 and 6 Ag-staining areas per diploid interphase in D. affinis, respectively between 1 and 4 areas in D. subobscura, may be regarded as being due to fusion of some but not all of the transcribing NORs, rather than as the result of variation in the number of genetically active NORs per cell within individuals, since the latter parameter has been proved to be constant in human cells (Markovic et al., 1978). That fusion between NORs does occur, has been established for human metaphases (Miller, D.A. et al., 1977).

III. 4. In Situ Hybridisation of Cloned Histone Genes

III.4.1. In Situ Hybridisation to the Polytene Karyotype of *D. affinis*

In contrast to the position of histone genes in the polytene karyotype of *D. melanogaster* (Pardue et al., 1978), two separate regions appear labelled in the genome of *D. affinis*, namely region 50B on chromosome C and region 106B on the long arm of chromosome E after in situ hybridisation of nick-translated pDmh 604 DNA to polytene chromosomes from salivary glands (Fig. 35). It is evident from the chromosome map of the polytene complement in *D. affinis* (Fig. 2) that both labelled regions are in centromere vicinity, similar to the position of histone sequences in region 39DE on the left arm of chromosome 2 in *D. melanogaster*.

After an exposure time of 7 days, 48 (85.71%) out of 56 nuclei, which allowed clear identification of region 50B and 106B, appeared labelled. 17 (30.36%) out of the 56 nuclei were labelled in region 106B and 50B, 13 nuclei (23.21%) showed exclusive label in region 50B, and 18 nuclei (32.14%) were labelled in region 106B only.

At a background level of almost 0, the relevant regions were considered as significantly labelled when they were covered by at least 3 silver grains each. A mean label intensity of 4.8 ± 1.8 silver grains was calculated for region 106B and of 5.2 ± 1.8 silver grains for region 50B. The frequencies of label intensity over both regions, 50B and 106B, are normally distributed (Fig. 36a and b). No hybrid formation was detected in control slides, that had not been subjected to the DNA denaturation treatment. As the appearance of polytene bands has become less distinct by the DNA denaturation treatment in 0.07N NaOH prior to

incubation in the hybridisation mixture, it is not possible to tell from the label distribution whether the position expands over the distal part of region 106B. It is worth noting that region 106B belongs to the chromosomal segment adjacent to the chromocenter that occasionally undergoes heteropycnosis (compare section III.1.3.). Also in region 50B it is for technical reasons not possible to locate the label distribution more differentially.

In the salivary gland squash preparations from D. melanogaster used as control, region 39DE appeared labelled at much higher density, that did not allow grain counting after an exposure time of 7 days (Fig. 38d), compared to D. affinis and D. subobscura. Region 39DE was identified according to the salivary gland chromosome map of Bridges (1935). This region appeared labelled in 191 nuclei (100%).

III.4.2. In Situ Hybridisation to the Polytene Karyotype of D. subobscura

In situ hybridisation of nick-translated pDmh 604 DNA to salivary gland chromosomes revealed autoradiographic label to be restricted to region 41C on chromosome U in Drosophila subobscura (Fig. 37 and 38a-c). Slides from two series were examined. In the first series, 91 nuclei allowed clear identification of region 41C according to the salivary gland chromosome map from Kunze-Mühl and Müller (1958), and in the second series, region 41C could be recognized in 48 nuclei. All 139 nuclei were labelled. In the control preparation, that had not been subjected to the DNA denaturation treatment in 0.07N NaOH, no hybrid formation could be detected.

To allow clear localization of silver grains within region 41C in the examined stock of D. subobscura, it was necessary to improve the salivary gland chromosome map of Kunze-Mühl and Müller (1958) between region 41B and 42A (Fig. 40B). In lactoacetic orcein squash preparations of salivary gland chromosomes from prepupae, a thin, faintly stained band proximal to the labelled band after in situ hybridisation of histone sequences was observed within region 41C. Occasionally, this band also showed up in toluidine blue-stained preparations, but it was never consistently found in any stained preparation. The proximal thin band in region 41C often appeared puffed in prepupae. The thicker band in region 41C, which was labelled in my autoradiographs, stained more intensely in orcein-stained preparations than was indicated in the chromosome map of Kunze-Mühl and Müller. In region 42A, the proximal band appeared thinner than the one distal in contrast to the indications in Kunze-Mühl and Müller's map, where the distal band in region 42A shows up less intensely than the proximal band. It is known, that the various pretreatments of slides in hybridisation experiments such as ribonuclease treatment and denaturation of chromosomal DNA in 0.07N NaOH may cause artifactual changes in band morphology of polytene chromosomes. In my autoradiographs, the distal band in region 41D often appeared swollen and thicker than in orcein-stained preparations; I assume this to be due to the pretreatment of slides prior to hybridisation.

The distribution of silver grains over region 41C is either confined to the distal band within the region (Fig. 38a), or proceeds over the distal interband, leading to region 41D (Fig. 38b and c). The latter pattern was observed in most nuclei examined, though in many cases it was difficult to attribute the autoradiographic label precisely to the distal band and to the adjacent interband within the region due to low autoradiographic resolution.

The mean label intensity was determined in slides that were exposed for 7 days and in those having been left to expose for 13 days (Fig. 41a and b). In nuclei exposed for 13 days, the mean number of silver grains over region 41CD amounts to 13.3 ± 4.04 , whereas nuclei exposed for 7 days exhibit at the same region a mean grain number of 7 ± 2.4 .

The mean label intensities over regions 106B in D. affinis and 41C in D. subobscura significantly differ from each other at the 5%-level according to the F-test after 7 days of autoradiographic exposure, while the difference in label intensity between regions 50B in D. affinis and 41C in D. subobscura is not significant. According to the z-test, also the percentage of labelled nuclei significantly differs at the 0.3%-level between D. affinis and D. subobscura.

IV. DISCUSSION

IV. 1. General Discussion

There are two ways to explain the sex ratio phenomenon genetically. The inversions on the X-chromosome may cause position effects. That means, that only gene activity is affected and not the gene itself. The other possibility is the occurrence of one or more gene mutations which interrupt the normal differentiation of Y-bearing spermatids. However, since the sex ratio phenomenon is strictly correlated with a particular combination of X-linked inversions in both Drosophila species examined, the inversion must have a function for the sex ratio phenomenon.

The presented results on the location of histone genes in the karyotype of Drosophila affinis and Drosophila subobscura give evidence that histone genes are linked to autosomes and not to X-chromosomes in both species. Therefore, the histone loci are unlikely to influence directly failure of spermiogenesis in Y-bearing sperm of sex ratio males.

The AgAS technique revealed transcribing NORs in the male mitotic chromosomes from D. subobscura in the first autosome pair, in the centromere proximity of the X-chromosome, and on the heterochromatic Y-chromosome. As shown by Giemsa C-staining, the location of the NORs on the X-chromosome coincides with the position of heterochromatin. As no heterochromatin can be detected in the polytene salivary gland nuclei from D. subobscura, the presence of latitudinal multiple copies of those ribosomal sequences can be excluded.

As the most proximal breakage site of the sr gene arrangement $A_{2+3+5+7}$ lies between region 1B and 1C, i.e. in the polytenized euchromatic part of the chromosome A (Jungen, 1968a), the ribosomal sequences are not transposed by the inversions.

Also in Drosophila affinis, X-linked inversions were found in centromere proximity. Different from Drosophila subobscura, in this species heterochromatin polytenizes in salivary gland nuclei. However, in the sr gene arrangements XS-I, XL-II and XS-II, XL-IV, the proximal breakage sites of the inversions are located distally to the heterochromatin, thus leaving the position of ribosomal cistrons, as detected in the Ag-stained mitotic X-chromosome, unchanged.

In both species, the observation made in Ag-stained polytene nuclei still leaves the possibility open that some more NORs are distributed along the X-chromosome and on autosomes. At present this possibility remains purely speculative, as the significance of Ag-positive bands of polytene chromosomes is still obscure.

IV. 2. Analysis of the Polytene Karyotype of *D. affinis*

According to the salivary gland chromosome map presented in this study, the polytene karyotype of *Drosophila affinis* is composed of 1164 light-microscopically visible bands. As previously stated, this figure most probably represents an underestimate of the real band number, which is due to the difficulty to recognize closely adjacent bands as being separated. Bands that were likely to represent double bands were counted as broad single bands if no interband between the two bands could be seen in the lactoacetic orcein stained preparations.

The fact, that the collective chromocenter exhibits all morphological characteristics of β -heterochromatin according to the definition of Heitz (1934) and does not possess any visible α -heterochromatin in its center, distinguishes *Drosophila affinis* from *Drosophila melanogaster* and other *Drosophila species* (for references see Beermann, 1962), that exhibit a chromocenter in their salivary gland nuclei. In these species, the α -heterochromatin is known as "not growing" in the course of polytenization (Heitz, 1934), thus, representing underreplicated chromatin (Rudkin, 1969). In *Drosophila melanogaster*, the Y-chromosome in male karyotypes and the centromeres of all chromosomes within the polytene complement constitute this underrepresented α -heterochromatin, while β -heterochromatin evolves by heteropycnosis of partly polytenized centromere near segments (Beermann, 1962).

Satellite DNA, located in the α -heterochromatin, was shown to replicate at least a few times in the course of polytenization in D. melanogaster, as demonstrated by CsCl equilibrium ultracentrifugation of DNA from diploid and polytene cells (Gall et al., 1971). In the case of D. affinis, the absence of clearly recognizable α -heterochromatin in the collective chromocenter may be interpreted as follows: in contrast to the situation in D. melanogaster, where the α -heterochromatin content in polytene nuclei is slightly increased compared to diploid nuclei, the heterochromatic Y-chromosome and the centromeres do not undergo further replication steps at all in salivary gland nuclei from D. affinis. Though there might occur some α -heterochromatin in the collective chromocenter, it would appear too small within the prominent β -heterochromatic portion to be detected by light microscopical examination.

An interesting finding is the observation of a prominent intercalary β -heterochromatic block in region 135B on the short arm of chromosome X in D. affinis. According to Beermann (1962), large intercalary heterochromatic blocks rarely appear in polytene chromosomes, and usually consist of α -heterochromatin as revealed in Acricotopus lucidus. Intercalary heterochromatin more often appears as "weak points", i.e. as sites highly sensitive to mechanical stress as observed in Drosophila melanogaster (Bridges, 1935) and D. pseudoobscura (Dobzhansky, 1944), or as constrictions found in Phryne (Wolf, 1957). β -heterochromatin in the short arm of chromosome X in D. affinis is missing in the illustrations published by Miller (1977) for the short arm of chromosome X from animals originating from Nebraska, and it also has never been published previously. Its presence in the stock used in my studies might be led back to the ancient occurrence of a peri-

centric inversion with one breakage site located in the centromere and another in intercalary position, immediately distal to region 135B.

The variation in banding patterns of some regions, such as region 2Aa-2Ab and 6Ac-6Bb on the short arm of chromosome B, 101Ba-101Bd on the short arm of chromosome E, 129A on the long arm of chromosome E as well as 106Aa-106Bc, occasionally appearing as heterochromatic part of the collective chromocenter, cannot be regarded as genetic and consequently inheritable modifications of the polytene karyotype, as all modifications may occur within the same salivary gland. They rather reflect either variable responses to the fixation treatment in ethanol/acetic acid (3:1) and subsequently in 50% acetic acid, or morphological changes in the relevant regions prior to pupation, when the salivary glands normally undergo lysis.

The puffs that regularly occur in the polytene karyotype of D. affinis and that are shown in the chromosome map (Fig. 2), constitute the puffing pattern as it is observed in the prepupal stage only. Fewer puffs are visible within the third larval instar, but the karyotype has not been analysed at this stage, as the preparation of salivary gland chromosomes is facilitated in prepupae.

Iv. 3. Localization of Nucleolus Organizing Regions (NORs) in *D. affinis*
and *D. subobscura*

In the course of the last few years, ammoniacal silver-staining (AgAS-technique) of chromosomes has become a common method for the specific detection of transcriptionally active NORs in animal- (Miller, D.A. et al., 1976; Miller, U.J. et al., 1976; Czaker, 1978) and plant chromosomes (Schweizer and Ambros, 1979), and was at first mainly used in mammals (Goodpasture and Bloom, 1975; Yosida, 1978; Noel et al., 1978) with particular reference to the location of NORs in the human chromosome complement (Archidiacono et al., 1977; Denton et al., 1977; Abe et al., 1978; Buys et al., 1978; Howell et al., 1978). Howell (1977) showed in meiotic cricket chromosomes that preferential silver-staining of transcribing NORs is based on reduction of the silver ion by acidic proteins that rapidly associate with freshly transcribed RNA. This evidence was confirmed by Olert et al. (1979), who observed silver ion reduction by the carboxyl groups of acidic proteins in cultured human fibroblasts and lymphoblasts. Eventually, the AgAS-technique was applied on polytene chromosomes of *Rhynchosciara hollaenderi* (Stocker et al., 1978). The authors observed two types of silver-staining patterns depending on the staining conditions, namely an Ag-banding pattern or

a "nucleolar-specific" Ag-staining pattern. Some of the silver-positive chromosomal bands coincided with the position of ribosomal genes as detected by in situ hybridisation of ^3H -rRNA to the polytene chromosomes (Pardue et al., 1970). Additional heterochromatic bands rich in satellite DNA also stained Ag-positively. The authors attribute these additionally staining heterochromatic bands to the presence of some acidic proteins other than observed at rDNA sites of mitotic human (Olert et al., 1979) or meiotic cricket cells (Howell, 1977).

Varley and Morgan (1978) did not find the claimed specificity of the AgAS-technique for genetically active NORs when staining lampbrush chromosomes of Triturus cristatus carnifex. The authors compared the Ag-staining pattern in loops and non-loop like structures with the labelling pattern obtained by Hennen et al. (1975) after in situ hybridisation of ^{125}I -rDNA to lampbrush chromosomes of the same species. None of these labelled loops stained with silver, even not sites attached to nucleoli or secondary constrictions, despite the presence of specifically Ag-staining extra-chromosomal nucleoli.

The presented studies on ammoniacal silver-staining of salivary gland chromosomes in D. affinis and D. subobscura resulted in characteristic silver-staining patterns consisting of approximately 50 positively staining bands distributed over the whole complement in both species. The consistency of the staining pattern was shown in regions 202B and 206B in D. affinis and in regions 15C, 16B and 16C in D. subobscura, both chromosomal segments being X-linked. In both species, nucleolar Ag-staining was observed. Comparing these results, however, with the labelling pattern obtained after in situ hybridisation of ^3H -rRNA from

D. melanogaster, the coincidence of ^3H -labelled and Ag-positive sites is limited to the rDNA representing a part of the nucleolus. No firm conclusions can be drawn from these observations with respect to the significance of the Ag-positive chromosomal bands. Yet, the consistency of their appearance together with the demonstration that puffs - known as transcriptionally active sites associated with non histone proteins (Lezzi, 1967) - do not stain differentially by ammoniacal silver allow the following suggestions: possibly, Ag-positive bands partly represent transcribing NORs, that cannot be detected by in situ hybridisation of ^3H -rRNA due to too low a repetition degree of ribosomal cistrons within each NOR. This assumption is supported by the likely occurrence of the underreplication of rDNA in D. affinis and D. subobscura salivary gland chromosomes, as has been shown for D. melanogaster (Spear and Gall, 1973) and D. hydei (Hennig and Meer, 1971) (for further references see Nagl, 1978). Underreplication of ribosomal sequences in the polytene karyotype would result in a relative decrease of rDNA copies as compared to the mitotic karyotype, consequently, rendering the detection of these genes even more difficult by in situ hybridisation. Nonetheless, they would be detected by Ag-staining, as the amount of acidic proteins at the transcribing NORs would be above the limit of the resolution of this technique in polytene chromosomes. In addition to the ribosomal cistrons, positively silver-staining polytene bands might represent specific regions other than NORs but possibly associated with similar acidic proteins as had been demonstrated at transcribing NORs.

More specific evidence on the location of transcriptionally active NORs by AgAS-staining is attained in diploid nuclei from larval brain ganglia in D. affinis and D. subobscura. The control preparations from the same material in D. melanogaster, where the position of NORs is known to be restricted to the sex chromosomes (Ritossa and Spiegelmann, 1965), clearly exhibited NOR-activity at the respective sites. Neither in D. affinis nor in D. subobscura Ag-positive sites are distributed over the whole mitotic complement as compared to the polytene chromosome set, but are restricted to the sex chromosomes and to another acrocentric chromosome pair in both species. In D. affinis mitoses, two additional transcribing NORs appeared in the two dot-like chromosomes.

The Ag-staining pattern in the mitotic chromosomes of D. hydei did not reveal Ag-positive staining in the position of NORs (Beck and Srdic, 1979), which had been previously located in a terminal region of the X-chromosomal heterochromatic arm as well as on the short arm of the Y-chromosome by in situ hybridisation of rRNA (Hennig et al., 1975). However, the Ag-technique that the former authors used represents a modification of the AgAS -technique usually applied, in that the preparations were incubated in a 50% AgNO₃-solution at 60°C for 4-20 hrs. without subsequent development in ammoniacal AgNO₃ plus 1% formaldehyde. Preliminary experiments, that had been performed on neuroblasts of D. affinis and D. subobscura, had shown that this modification did not result in consistently Ag-dark regions in the mitotic chromosome complement but in slightly darker staining of variable intensity of heterochromatin as compared to euchromatic regions.

In neuroblast chromosomes from D. affinis, D. subobscura and D. melanogaster, no secondary constrictions can be seen at the position of NORs, as it is the case in most plant karyotypes and in some mammalian nucleolus organizing chromosomes (Elsevier and Ruddle, 1975) or amphibian NO-chromosomes (Macgregor and Mizuno, 1976; Barsacchi-Pilone et al., 1977). However, as stated in section III.3.2., preferential silver deposition at NORs can clearly be distinguished from Ag-positive staining of constitutive heterochromatin, which stains in various intensity and never as intensely as transcriptionally active NORs.

While in situ hybridisation experiments failed to reveal any ribosomal cistrons at the respective chromosomal sites in mitoses, presumably as the consequence of too low an efficiency in the formation of silver grains in the autoradiographs, the detection of transcribing NORs by ammoniacal silver-staining can be explained by the progressive accumulation of acidic proteins associated with rRNA. This consequently means that in NOR-specifically Ag-staining cells, the sensitivity of the staining method exceeds the one of the in situ hybridisation technique. The accumulation of nucleolar material at the NORs within a mitotic chromosome complement is comparable to the formation of micronucleoli, that tend to fuse, eventually forming the nucleolus. The comparison of the mean label intensity over diploid interphases in D. affinis, D. subobscura and D. melanogaster with the respective mean number of Ag-positive areas per diploid interphase may illustrate the higher sensitivity of the AgAS-technique in detecting transcribing ribosomal genes. No significant differences are found in mean label intensity between the species examined, despite marked differences in the number of transcribing NORs.

This indicates that D. melanogaster, possessing 2 NORs in a diploid cell, possibly hybridises with relatively more rRNA from the same species, as compared to D. affinis and D. subobscura that exhibit 6-7, respectively 4 transcriptionally active NORs in their diploid interphases let alone some possible undetected inactive NORs in interstitial positions. The differences in the mean number of Ag-positive areas per diploid interphase, though, are correlated with the differences in the number of active NORs per metaphase between species.

IV. 4. Localization of Histone Genes in the Polytene Karyotypes of D. affinis and D. subobscura

Histone genes appear in the eukaryotic genome as reiterated repeat units, each containing sequences coding for all the five histone fractions separated from each other by spacer sequences of unknown function. Particularly extensive studies on the molecular organization of histone genes have been performed in Drosophila melanogaster (for review see Kedes, 1976) and in several sea urchin species (Birnstiel et al., 1972). In interpreting the results obtained by in situ hybridisation of nick-translated cloned histone sequences from D. melanogaster to the polytene karyotypes of D. affinis and D. subobscura, it seems important to consider some main aspects concerning the organization of histone gene repeat units.

In D. melanogaster, histone genes are repeated approximately 50 times per haploid genome in tandem array, each repeat unit consisting of histone coding sequences in the following arrangement: H1-H3-H4-H2a-H2b

(Karp and Hogness, 1976). This cluster of histone gene repeats falls into region 39DE on the left arm of chromosome 2 in salivary gland chromosomes (Pardue et al., 1977). Spacer sequences, separating the histone coding sequences within one repeat unit, much differ in length from those found in sea urchin histone gene repeats. In Strongylocentrotus purpuratus, the spacers vary in length between 600 and 1200 bp (Cohn et al., 1976). In Psammechinus miliaris, the spacers have been found to be AT-rich as shown by denaturation experiments in formaldehyde (Portmann et al., 1976). In D. melanogaster, four spacer sequences are 0-250 bp in length each, whereas the spacer between H1 and H3 comprises 1200 bp. This long spacer appears as the only AT-rich sequence within the repeat unit, as revealed by electron-microscopic studies. When the D. melanogaster repeat unit was spread in 80% formamide, the long spacer sequence denatured exclusively (see Lifton et al., 1978).

The molecular organization of the histone gene repeat unit in D. affinis and D. subobscura has not been approached yet, and consequently, no data are known about length distribution of spacers in their histone gene repeat units and about histone DNA sequence divergence between these two species and D. melanogaster. It might be argued that the significant differences in mean label intensity over regions 106B and 50B in D. affinis, over 41C in D. subobscura and over 39DE in D. melanogaster after in situ hybridisation of cloned D. melanogaster histone genes, as well as the difference in the percentage of labelled nuclei between D. affinis and D. subobscura reflect sequence heterogeneity between histone DNA sequences from D. affinis, D. subobscura and D. melanogaster. This would be comparable to sequence divergence in histone

genes between sea urchins such as the closely related genera Paracentrotus and Psammechinus (Weinberg et al., 1972) where 40-50% of the mRNA coding for various histone fractions was able to anneal to histone DNA sequences in reciprocal cross hybridisation experiments. The authors consider heterogeneity of histone DNA sequences between species to be the result of mutational drift during evolution.

An alternative interpretation of the exclusive labelling of regions 106B and 50B in D. affinis and 41C in D. subobscura is the possibility of hybrid formation between the AT-rich 1200 bp spacer sequence, which separates the sequences coding for the H1- and the H3 fraction, and any AT-rich sequences within the genome of D. affinis and D. subobscura. However, any hybrid formation of that unspecific character would most likely also have occurred in the control salivary gland preparations from D. melanogaster, leading to additional labelled regions outside 39DE. Such regions have not been detected in any polytene nuclei examined in D. melanogaster.

Based on the same criteria, any unspecific association of AT-rich or GC-rich sequences within the pDmh 604 plasmid to respective other sequences than comprised in the histone genes from D. affinis and D. subobscura, leading to additional labelled sites in the preparations examined, may be excluded.

There is evidence in sea urchins that the composition of histone proteins exhibits developmental variations within an individual. In D. melanogaster, several variants have been found for the H1 histone fraction (Newrock et al., 1978). Lifton et al. (1978) postulate that each chromomere within region 39DE in D. melanogaster comprises a

specific set of identical histone gene repeat units, all of which are simultaneously activated by puffing of the relevant bands. If this hypothesis bears truth, one might speculate for the histone genes in D. affinis and D. subobscura that other histone fractions beside H1 exhibit variants in the course of ontogenesis, which are reflected in heterogeneity of the sequences coding for the respective histone fractions. In view of thereby increased differences in histone gene sequences between D. affinis and D. subobscura on one hand and D. melanogaster on the other hand, the differences in label distribution over the relevant regions in the species examined seem to be understandable. Alternatively, a possible difference in repetition degree of histone genes between the species examined may be taken in account as the cause of label distribution over fewer bands in D. affinis and D. subobscura as compared to D. melanogaster.

The detection of histone gene clusters in the respective regions in D. affinis and D. subobscura does not exclude the possibility of single or low numbers of histone genes being present in other positions in the genome than revealed in the present study. The conditions used in in situ hybridisation experiments would not allow to detect these sites.

The evidence that in D. affinis histone DNA sequences are located in two unlinked regions, both in the vicinity of the chromocenter in the polytene karyotype, leads to the following speculations. The presence of two unlinked histone gene clusters could be considered as the result of a reciprocal translocation between chromosome E and chromosome C, that had taken place in the course of species diversification within

the D. affinis subgroup. One event of breakage and reunion would have occurred within the histone gene cluster, which consequently got split up into two unlinked parts. Since the long arms of chromosomes X and B and the short arm of chromosome E have been homologised between D. affinis, D. athabasca and D. algonquin, regarding specific inverted segments in the three species (Miller, 1977), one might assume that the postulated reciprocal translocation would also be reflected in these other species within the D. affinis subgroup.

The fact that regions 106B and 50B are situated near the chromocenter can be interpreted when considering the results obtained by Baimai (1977), who observed the presence of an extra heterochromatic portion in intercalary position in the mitotic and polytene inverted chromosome 3 in D. rectilia. One breakpoint of the inverted segment occurred in the vicinity of constitutive heterochromatin, leading to the authors hypothesis that chromosomal sites, that originally were located near constitutive heterochromatin, cause the production of additional heterochromatin when transposed into another part of the chromosome by an inversion. Applied to the positions of regions 106B and 50B in D. affinis, a transposition of these two regions to more distal parts on the respective chromosome arms might have caused heterochromatisation of these regions and consequently inactivation of histone genes, against which selective pressure is likely to be directed.

The histone gene cluster in the polytene karyotype of D. subobscura, region 41C on chromosome U, falls into a chromosomal portion that is involved in various combinations of partly superimposed structural rearrangements, that build up part of an inversion polymorphism. Each combination transferred region 41C into another new position on chromosome U. Such combinations of inversions affecting the position of region 41C are: U_1 , U_{1+2} , U_{1+2+4} , U_{1+2+6} and U_{1+2+8} (Sperlich, 1961; Sperlich and Kunze-Mühl, 1963; Goetz, 1965; Jungen, 1968) as well as U_5 (Kunze-Mühl and Müller, 1958). The authors clearly showed that specifically rearranged karyotypes occur in distinct frequencies, which correlate with specific changes in various ecological factors, suggesting natural discrimination against random combinations of structural rearrangements (Sperlich, 1963). The close genetic environment of region 41C, that has been shifted to various positions on chromosome U, remains unchanged in any inverted karyotype, as there is no breakage site of inversions placed in or immediately adjacent to 41C. The nearest breakage site distal to 41C is induced by inversion U_8 and coincides with region 44D/45A. The nearest breakage site proximal to 41C falls into region 40D/41A and is induced by inversion U_5 . Therefore, the possibility of breakage within a cluster of histone gene repeat units, building up two separate clusters within the genome, can be excluded.

In conclusion, histone DNA sequences being localized in region 41C in the polytene karyotype of D. subobscura might be considered as one type of gene family that is subject to strong selective elimination in any other close genetic environment than given by the succession of genes between regions 41A and 44D on chromosome U.

V. REFERENCES

- Abe, T., Kanai, K., Misawa, S. and Nakagome, Y.: Silver-staining for the analysis of rearrangements of human acrocentric chromosomes. Proc. Jap. Acad. Sci. 54, 451-454 (1978)
- Archidiacono, N., Caroa, A.D., Ferraro, M., Pelliccia, F., Rocchi, A. and Rocchi, M.: Localization of nucleolar organizers in man by specific staining. Atti Ass. Gen. Ital. 22, 115-116 (1977)
- Atherton, D. and Gall, J.G.: Salivary gland squashes for in situ nucleic acid hybridisation studies. D.I.S. 49, 131-132 (1972)
- Baimai, V.: Chromosomal polymorphisms of constitutive heterochromatin and inversions of Drosophila. Genetica 85, 85-93 (1977)

- Barsacchi-Pilone, G., Nardi, I., Andronico, F. and Battistoni, R.:
Chromosomal location of the rRNA genes in Triturus
vulgaris meridionalis (Amphibia, Urodela).
Chromosoma (Berl.) 63 127-134 (1977)
- Baumberger, H.: Chromosomenzahlbestimmungen und Karyotypanalysen bei
den Gattungen Anemone, Hepatica und Pulsatilla.
Ber. Schweiz. Bot. Ges. 80, 17-95 (1970)
- Beck, H. and Srdic, Z.: Heterochromatin in mitotic chromosomes of
Drosophila hydei.
Genetica 50, 1-10 (1979)
- Beermann, W.: Riesenchromosomen.
Protoplasmatologia (Alfert, M., Bauer, H., Harding, C.V.,
eds.) Wien, Springer Verlag 1962
- Birnstiel, M.L. and Chipchase, M.: Current work on the histone operon.
TIBS 2, 149-152 (1977)
- Birnstiel, M.L., Weinberg, E.S. and Pardue, M.L.: Evolution of 9S
mRNA sequences. In: Molecular Cytogenetics (Hamkalo, B.A.
and Papaconstantinou, J., eds.).
New York Plenum Publ. Corp., 75-93 (1973)

Bridges, C.B.: Salivary chromosome maps.

J. Hered. 26, 60-64 (1935)

Buyts, C.H.C.M., Osinga, J., Gouw, W.L. and Anders, G.J.P.A.:

Rapid identification of chromosomes carrying silver-stained nucleolus organizing regions. Application to a case of 21/21 Robertsonian translocation.

Human Gen. 44, 173-180 (1978)

Cohn, R.H., Lowry, J.C. and Kedes, L.H.: Histone genes of the sea

urchin Strongylocentrotus purpuratus cloned in E. coli: order, polarity and strandedness of the five histone coding and spacer regions.

Cell 9, 147-162 (1976)

Czaker, R.: Silver-staining in transcriptionally active NORs of meiotic and mitotic cells in Achaeta domesticus (L.) (Orthoptera).

Chromosoma (Berl.) 68, 187-193 (1978)

Denton, T.E., Brooke, W.R. and Howell, W.M.: A technique for the simultaneous staining of both nucleolar organizer regions and kinetochores of human chromosomes with silver.

Stain Technol. 52, 311-313 (1977)

Dobzhansky, Th.: Chromosomal races in Drosophila pseudoobscura and Drosophila persimilis.

In: contributions to the Genetics, Taxonomy and Ecology of Drosophila pseudoobscura and Its Relatives (Dobzhansky, Th. and Epling, C., eds.).

Carnegie Inst. of Wash. Publ. 554, 47-144 (1944)

Dubler-Hänggi, S.: Spermatogenese von Drosophila subobscura bei Kontroll- und sex ratio-Tieren.

Dissertation an der Universität Zürich (1977)

Elsevier, S.M. and Ruddle, F.H.: Location of genes coding for 18S and 28S ribosomal RNA within the genome of Mus musculus.

Chromosoma (Berl.) 52, 219-227 (1975)

Gall, J.G., Cohen, E.H. and Atherton, D.D.: The satellite DNAs of Drosophila virilis.

Cold Spring Harb. Symp. Quant. Biol. 38, 417-421 (1971)

Goetz, W.: Chromosomaler Polymorphismus in einem Muster von Drosophila subobscura aus Marokko, mit Darstellung der Heterozygotieverhältnisse als Heterozygotiediagramm.

Z. VererbungsL. 97, 40-45 (1965)

Goetz, W.: Untersuchungen über den chromosomalen Strukturpolymorphismus in kleinasiatischen und persischen Populationen von Drosophila subobscura Collin.

Mol. Gen. Gen. 100, 1138 (1967)

Goodpasture, C. and Bloom, S.E.: Visualization of nucleolar organizer regions in mammalian chromosomes using silver-staining. Chromosoma (Berl.) 53, 37-50 (1975)

Hauschteck-Jungen, E., Jungen, H. and Müller, M.: Karyotyp und Meiose bei wild- und sex ratio-Männchen von Drosophila subobscura.

Rev. Suisse Zool. 79, 298-305 (1972)

Hauschteck-Jungen, E. and Maurer, B.: Sperm dysfunction in sex ratio-males of Drosophila subobscura.

Genetica 46, 495-477 (1976)

Heitz, E.: Ueber α - und β -heterochromatin sowie Konstanz und Bau der Chromomeren bei Drosophila.

Biol. Zbl. 54, 588-609 (1934)

Hennen, S., Mizuno, S. and Macgregor, H.C.: In situ hybridisation of ribosomal DNA labelled with ¹²⁵I-iodine to metaphase and lampbrush chromosomes from newts.

Chromosoma (Berl.) 50, 349-369 (1975)

- Hennig, W. and Meer, B.: Reduced polyteny of ribosomal RNA cistrons
in giant chromosomes of Drosophila hydei.
Nature (Lond.) New Biol. 233, 70-72 (1971)
- Hennig, W., Link, B. and Leoncini, O.: The location of the nucleolus
organizer regions in Drosophila hydei.
Chromosoma (Berl.) 51, 57-63 (1975)
- Howell, W.M.: Visualization of ribosomal gene activity: silver stains
proteins associated with rRNA transcribed from oocyte
chromosomes.
Chromosoma (Berl.) 62, 361 - 367 (1977)
- Howell, W.M., Howard-Peebles, P.N., Block, B.M. and Stoddard, G.R.:
Silver stain reveals nucleolus organizer regions on a
satellited Y_q-chromosome.
Human Gen. 42, 245-250 (1978)
- Jungen, H.: Inversionspolymorphismus in tunesischen Populationen von
Drosophila subobscura Collin.
Arch. J.K. Stiftung XLIII, 1-54 (1968a)
- Jungen, H.: "Sex ratio" in natürlichen Populationen von Drosophila
subobscura.
In: 28. J.b. d. Schweiz. Ges. Vererb.f.
Arch. J.K. Stift. XLIII, 52-57 (1968b)

Karp, R. and Hogness, D.S.: Isolation and mapping of histone genes
contained in cloned segments of Drosophila DNA.

Fed. Proc. 35, 1623-1626 (1976)

Kedes, L.H.: Histone messengers and histone genes.

Cell 8, 321-332 (1976)

Kunze-Mühl, E. and Müller, E.: Weitere Untersuchungen über die chromosomale Struktur und die natürlichen Strukturtypen von Drosophila subobscura Collin.

Chromosoma (Berl.) 9, 559-570 (1958)

Lezzi, M.: RNS- und Proteinsynthese in Puffs isolierter Speicheldrüsenchromosomen von Chironomus.

Chromosoma (Berl.) 21, 72-88 (1967)

Lifton, R.P., Goldberg, M.L., Karp, R.W. and Hogness, D.S.:

The organization of the histone genes in Drosophila melanogaster: Functional and evolutionary implications.

Cold Spring Harb. Symp. Quant. Biol. 42, 1047-1051
(1978)

Macgregor, H.C. and Mizuno, S.: In situ hybridisation of "nick-translated" ^3H -ribosomal DNA to chromosomes from salamanders.

Chromosoma (Berl.) 54, 15-25 (1976)

Markovic, V.D., Worton, R.G. and Berg, J.M.: Evidence for the inheritance of silver-stained nucleolus organizer regions.

Human Gen. 41, 181-187 (1978)

Miller, D.A., Tantravahi, R., Dev, V.G. and Miller, O.J.:

Frequency of satellite association of human chromosomes is correlated with the amount of Ag-staining of the nucleolus organizer region.

Am. J. Human Gen. 29, 490-502 (1977)

Miller, D.A., Dev, V.G., Tantravahi, R., Croce, C.M. and Miller, O.J.:

Human tumor and rodent-human hybrid cells with an increased number of active human NORs.

Cytogen Cell Gen. 21, 33-41 (1978)

Miller, D.D.: Salivary gland chromosome variation in the D. affinis subgroup. VI. Comparison of X, B and E chromosome patterns in D. athabasca and five related species.

J. Hered. 68, 105-113 (1977)

Miller, D.D. and Roy, R.: Further study of variation of the Y-chromosome of D. affinis subgroup species.

D.I.S. 39, 117 (1964)

Miller, O.J., Miller, D.A., Tantravahi, R. and Dev, V.G.:

Nucleolus organizer activity and the origin of Robertsonian translocation.

Cytogen. Cell Gen. 20, 40-50 (1978)

Nagl, W.: Endopolyploidy and polyteny in differentiation and evolution.

North Holl. Publ. Comp. 1978

Newrock, K.M., Alfageme, C.R., Nardi, R.V. and Cohen, L.H.:

Histone changes during chromatin remodelling in embryogenesis.

Cold Spring Harb. Symp. Quant. Biol. 42, 421-431
(1978)

Noel, B., Quack, B. and Benezech, M.: Le bivalent sexuel des mammifères observé par marquage argentique au stade pachytène.

Ann. Génét. 21, 83-87 (1978)

Olert, J., Sawatzki, G., Kling, H. and Gebauer, J.: Cytological and histochemical studies of the selective silver-staining of nucleolus organizer regions (NORs).

Histochem. 60, 91-99 (1979)

Pardue, M.L., Gerbi, S., Eckhardt, R.A. and Gall, J.G.:

Cytological localization of DNA complementary to ribosomal RNA in polytene chromosomes of Diptera.

Chromosoma (Berl.) 29, 268-290 (1970)

Pardue, M.L. and Gall, J.G.: Nucleic acid hybridisation to the DNA of cytological preparations.

In: Methods of Cell Biology X, (Prescott, D.M., ed.), 1-16 (1975)

Pardue, M.L., Kedes, L.H., Weinberg, E.S. and Birnstiel, M.L.:

Localization of sequences coding for histone mRNA in the chromosomes of D. melanogaster.

Chromosoma (Berl.) 63, 135-151 (1977)

Portmann, R., Schaffner, W. and Birnstiel, M.L.: Partial denaturation mapping of cloned histone DNA from the sea urchin Psammechinus miliaris.

Nature 264, 31-34 (1976)

Ritossa, F.M. and Spiegelmann, S.: Localization of DNA complementary to ribosomal RNA in the nucleolus organizer region of D. melanogaster.

Proc. Nat. Acad. Sci. (Wash.) 53, 737-745 (1965)

- Rogers, A.W.: Techniques of autoradiography.
Elsevier Publ Comp., Amsterdam-London-New York 1967
- Rudkin, G.T.: Non-replicating DNA in Drosophila.
Gen. Suppl. 61, 227-237 (1969)
- Schweizer, D. and Ambros, P.: Analysis of nucleolus organizer regions (NORs) in mitotic and polytene chromosomes of Phaseolus coccineus by silver-staining and Giemsa C-banding.
Plant Syst. Evol. 132, 27-51 (1979)
- Spear, B.B. and Gall, J.G.: Independent control of ribosomal gene replication in polytene chromosomes of D. melanogaster.
Proc. Nat. Acad. Sci. (Wash.) 70, 1359-1363 (1973)
- Sperlich, D.: Untersuchungen über den chromosomalen Polymorphismus einer Population von Drosophila subobscura auf den Liparischen Inseln.
Z. VererbungsL. 92, 74-84 (1961)
- Sperlich, D.: Non random association of inversions in D. subobscura.
D.I.S. 37, 130-131 (1963)
- Sperlich, D. and Kunze-Mühl, E.: Der chromosomale Polymorphismus einer Population von Drosophila subobscura auf der Insel Ustica im Vergleich mit anderen Insel- und Festlandstandorten.
Z. VererbungsL. 94, 94-100 (1963)

- Stocker, A.J.: Correspondence of silver banding with rRNA hybridisation sites in polytene chromosomes of Rhynchosciara hollaenderi.
Exp. Cell Res. 114, 429-434 (1978)
- Stocker, A.J., Fresquez, C. and Lentzios, G.: Banding studies on the polytene chromosomes of Rhynchosciara hollaenderi.
Chromosoma (Berl.) 68, 337-356 (1978)
- Stone, L.E.: Structure and variation of the salivary gland chromosomes in D. affinis.
D.I.S. 36, 119-120 (1962)
- Tokuyasu, K.T., Peacock, W.J. and Hardy, R.W.: Dynamics of spermiogenesis in Drosophila melanogaster. VII. Effects of Segregation Distorter (SD) chromosome.
J. Ultrastruct. Res. 58, 96-107 (1977)
- Varley, J.M. and Morgan, G.T.: Silver-staining of the lampbrush chromosomes of Triturus cristatus carnifex.
Chromosoma (Berl.) 67, 233-244 (1978)
- Voelker, R.A.: Preliminary characterisation of "sex ratio" and rediscovery and reinterpretation of "male sex ratio" in Drosophila affinis.
Genetics 71, 597-606 (1972)

Weinberg, E.S., Birnstiel, M.L., Purdom, I.F. and Williamson, R.:

Genes coding for polysomal 9S mRNA of sea urchins.

Conservation and divergence.

Nature (Lond.) 240, 225-228 (1973)

Wolf, B.E.: Temperaturabhängige Allozyklie des polytären X-Chromosoms

in den Kernen der Somazellen von Phryne cincta.

Chromosoma (Berl.) 8, 396-435 (1957)

Yosida, T.H.: A preliminary note on silver-stained nucleolar organizer regions
in the black and Norway rats.

Proc. Jap. Acad Sci. 54, 353-358 (1978)

VI. FIGURE LEGENDS

Fig. 1: Chromocenter from a polytene nucleus of D. affinis consisting of β -heterochromatin. Note the loosened granular appearance of the chromatin.

Lactoacetic-orcein. Bar=10 μ m

Fig. 2: Chromosome map of the polytene karyotype from salivary glands in D. affinis. The chromosome set is subdivided into 207 divisions, each of which consists of two subdivisions. The division number on each chromosome arm increases from the proximal to the distal end. The centromeric regions (Ch) of all chromosomes adhere and form the heterochromatic chromocenter. Capital letters refer to the chromosomes.

S: short arm, L: long arm, i Het: intercalary heterochromatin on the short arm of chromosome X.

Fig. 3: Variant banding patterns of region 2Aa-2Ab on BS.

a) The region appears as two closely adjacent thin dotted bands.

b) The same region appearing as a condensed darkly stained double band. Both variants can be found within the same salivary gland.

Ch: Chromocenter

Lactoacetic-orcein. Bar=10 μ m

Fig. 4: Variant banding patterns of region 6Ac-6Bb on BS.

a) The region consists of three condensed equidistant bands.

b) Discrete dotted band in 6Ac, followed by a condensed double band in 6Bb.

Lactoacetic-orcein. Bar=10 μ m

Fig. 5: Short arm of chromosome B.

◆ : weak point. Ch: chromocenter

Lactoacetic-orcein. Bar=10 μ m

Fig. 6: Long arm of chromosome B. The segment between the open arrows is inverted.

◆ : weak point. Ch: chromocenter.

Lactoacetic-orcein. Bar=10 μ m

Fig. 7: Chromosome C.

◆ : weak points. Ch: chromocenter.

Lactoacetic-orcein. Bar=10 μ m

Fig. 8: Variant banding patterns in region 101Ba-101Bd on ES.

a) 101Ba and 101Bb appearing as two discrete dotted bands followed by a more prominent condensed single band in region 101Bc.

b) 101Ba appearing as a dotted band followed by a double band in 101Bb and 101Bc.

Lactoacetic-orcein. Bar=10 μ m

Fig. 9: Short arm of chromosome E.

Ch: chromocenter.

Lactoacetic-orcein. Bar=10 um

Fig. 10: a) Region 106Aa-106Bc on EL included in the chromocenter (Ch).

b) 106Aa-106Bc remaining euchromatic. Heteropycnosis of 106Aa-106Bc does not occur in all nuclei within the same salivary gland.

Lactoacetic-orcein. Bar=10 um

Fig. 11: Long arm of chromosome E.

Ch: chromocenter.

Lactoacetic-orcein. Bar=10 um

Fig. 12: Chromosome F.

Ch: chromocenter.

Lactoacetic-orcein. Bar=10 um

Fig. 13: Short arm of chromosome X.

◀ : weak point. Ch: chromocenter. i Het: intercalary heterochromatin.

Lactoacetic-orcein. Bar=10 um

Fig. 14: Variant banding patterns in region 196Aa-196Bc on XL.

a) Distinct double band in 196A followed by two double bands in 196B.

b) Thin condensed band in 196Aa followed by two discrete dotted bands in 196Ab and 196Ba as well as two distinct double bands in 196Bb and 196Bc.

Lactoacetic-orcein. Bar=10 um

Fig. 15: Long arm of chromosome X.

a) Total arm.

b) Distal end of XL from another nucleus showing the banding pattern in region 200A more clearly.

◆ : weak point. Ch: chromocenter.

Lactacetic-orcein. Bar=10 μ m

Fig. 16: Chromosomal segment from XL, showing a small (+), a medium-sized (++) and a large (+++) puff.

+: a band is visible on the point of becoming decondensed or in a regressing state.

++: the chromatin in the puffed region is loosened, though the band structure is still recognizable.

+++ : decondensed chromatin; no bands are evident.

Lactacetic-orcein. Bar=10 μ m

Fig. 17: Label distribution over nucleolus from a polytene nucleus of D. subobscura after in situ hybridisation of ^3H -18+28S rRNA from D. melanogaster. Slides were hybridised with 10^5 cpm in $2 \times \text{SSC}$. Spec. act.: 1.2×10^6 cpm/ μ g. Denaturation of chromosomal DNA in 0.07N NaOH for 3-5 min. at room temperature. Exposition 21 days. Toluidine blue. Bar=10 μ m. Arrow points to toluidine blue darkly staining nucleolar area. The nucleolus is only partly labelled.

Fig. 18: Frequency distribution of label intensities after in situ hybridisation of ^3H -18+28S rRNA from D. melanogaster to nucleoli in polytene nuclei of D. subobscura. The values are corrected for background.

a) 21 days exposition.

b) 45 days exposition.

f: absolute frequency (number of nucleoli). n: number of silver grains.

Fig. 19: Label distribution over nucleolus from polytene nucleus of D. affinis. after in situ hybridisation of ^3H -18+28S rRNA from D. melanogaster. For experimental details see legend to Fig. 17. The nucleolus is partly labelled. Only part of the nucleolus appears stained (arrow).

Toluidine blue. Bar=10 μm

Fig. 20: Label distribution over nucleolus from polytene nucleus of D. melanogaster after in situ hybridisation of ^3H -18+28S rRNA from the same species. For experimental details see legend to Fig. 17. The stained part of the nucleolus is marked by an arrow.

Toluidine blue. Bar=10 μm

Fig. 21: Squash preparation of neuroblasts from D. subobscura.

Dip In: diploid interphase nuclei. Pol In: polyploid interphase nuclei.

Giemsa. Bar=10 μm

Fig. 22: Diploid neuroblast nucleus of a) D. affinis and b) D. subobscura in situ hybridised with ^3H -18+28S rRNA from D. melanogaster. Incubation in 1.7×10^5 cpm per slide in $2 \times \text{SSC}$. Spec. act.: 1.2×10^6 cpm/ug. Denaturation of chromosomal DNA in 0.07N NaOH for 3-5 min. at room temperature.

Note that the distribution of labelling may be restricted to the intensely stained area within the interphase nucleus (open arrows), while black arrows point to nuclei with average label intensity.

Exposition: 140 days. Toluidine blue. Bar=10 μm

Fig. 23: Frequency distribution of label intensities after in situ hybridisation of ^3H -18+28S rRNA from D. melanogaster to diploid interphases from larval brain ganglia of a) D. affinis, b) D. subobscura and c) D. melanogaster. The frequencies were determined from 100 cells in each species. The values are corrected for background.

f: absolute frequency (number of cells). n: number of silver grains.

Fig. 24: a) AgAS-staining pattern on the long arm of chromosome X from a polytene nucleus of D. affinis.

b) AgAS-staining pattern on the short arm of chromosome X of D. affinis. Ag-labelled sites are marked by an arrow.

Bar=10 μm

Fig. 25: AgAS-staining pattern of the polytene chromosome A (=X) of D. subobscura. Ag-labelled sites are marked by an arrow.
Bar=10 um

Fig. 26: Puffs in AgAS-stained polytene chromosomes from a) D. affinis and b) D. subobscura. Note the absence of Ag-positive sites in the puffed regions (arrows).
XL: long arm of chromosome X of D. affinis.
U: polytene chromosome U of D. subobscura.
Bar=10 um

Fig. 27: AgAS-staining pattern of a nucleolus from a salivary gland nucleus of D. affinis.
Bar=10 um

Fig. 28: Association between nucleolus and proximal end of the polytene chromosome A(=X) of D. subobscura. Note the absence of Ag-positive sites in region 1A.
Nu: nucleolus.
Bar=10 um

Fig. 29: Mitotic karyotype of D. subobscura obtained from larval brain ganglia squash preparations.
a) Giemsa C-staining pattern.
b) AgAS-staining pattern.
NORs are marked by an arrow.

Fig. 30: Mitotic karyotype of D. affinis obtained from neuroblast squash preparations.

a) Giemsa C-staining pattern.

b) AgAS-staining pattern.

NORs are marked by an arrow. The Y-chromosome in a) and b) seems to be out of proportion as compared to the X-chromosome, reflecting a polymorphism in size of the Y-chromosome as previously discovered by Miller and Roy (1964).

Fig. 31: Mitotic karyotype of D. melanogaster. obtained from neuroblast squash preparations.

a) Giemsa C-staining pattern

b) AgAS-staining pattern.

NORs are marked by an arrow. The chromosomes in a) are in prometaphase.

Fig. 32: Differential AgAS-staining pattern in diploid neuroblast interphases of a) D. affinis, b) D. subobscura and c) D. melanogaster. Note the presence of 6 (a), 4 (b) and 2 (c) Ag-labelled NORs (arrows).

Bar=10 μ m

Fig. 33: Differential AgAS-staining pattern in diploid neuroblast interphases from a) D. affinis, b) D. subobscura and c) D. melanogaster. Transcribing NORs seem to adhere to form a single Ag-labelled area (arrows).

Bar= 10 μ m

Fig. 34: Distribution of count frequencies referring to the number of Ag-labelled areas per diploid interphase in a) D. affinis, b) D. subobscura and c) D. melanogaster. In each species, a random sample of 210-220 cells was selected from three individuals including males and females.
f: absolute frequency (number of nuclei). n: number of Ag-labelled areas.

Fig. 35: Salivary gland nucleus of D. affinis in situ hybridised with ^3H -pDmh 604 plasmid DNA to detect the location of histone genes. Incubation in 8×10^4 cpm in 4 x SSC and 50% formamide. Spec. act.: 1.7×10^7 cpm/ug. Denaturation of chromosomal DNA in 0.07N NaOH for 3-5 min. at room temperature. Note label over region 106B on EL and over region 50B on chromosome C.
Exposition: 7 days. Toluidine blue. Bar=10 um

Fig. 36: Frequency distribution of label intensities over regions 106B (a) and 50B (b) in salivary gland nuclei of D. affinis after in situ hybridisation of nick-translated pDmh 604 plasmid DNA.
f: absolute frequency (number of nuclei). n: number of silver grains.
Exposition: 7 days.

Fig. 37: Chromosome U from a polytene nucleus of D. subobscura in situ hybridised with nick-translated pDmh 604 plasmid DNA. For experimental details see legend to Fig. 35. Note label over region 41C.
Toluidine blue. Bar= 10 um

Fig. 38: a) Region 41C in D. subobscura in situ hybridised with nick-translated pDmh 604 plasmid DNA. Note localization of silver grains being confined to the thick band within the region. Exposition: 7 days.
b) and c) The same region after an exposure time of 13 days. The distribution of silver grains expands from region 41C to 41D.
d) Region 39DE in D. melanogaster after in situ hybridisation of nick-translated pDmh 604 plasmid DNA. Exposition: 13 days. Note the high label intensity as compared to region 41C in D. subobscura. For experimental details see legend to Fig. 35.
Bar=10 um

Fig. 39: Region 41C on chromosome U in D. subobscura.
Lactoacetic-orcein. Bar=10 um

Fig. 40: a) Map of lactoacetic-orcein-stained region 41A-42A on chromosome U in D. subobscura from Kunze-Mühl and Müller (1958).

b) Modified map of the same segment.

Fig. 41: Frequency distribution of label intensities over region 41C in salivary gland nuclei of D. subobscura after in situ hybridisation of nick-translated pDmh 604 plasmid DNA.

a) Exposition: 7 days. b) Exposition: 13 days.

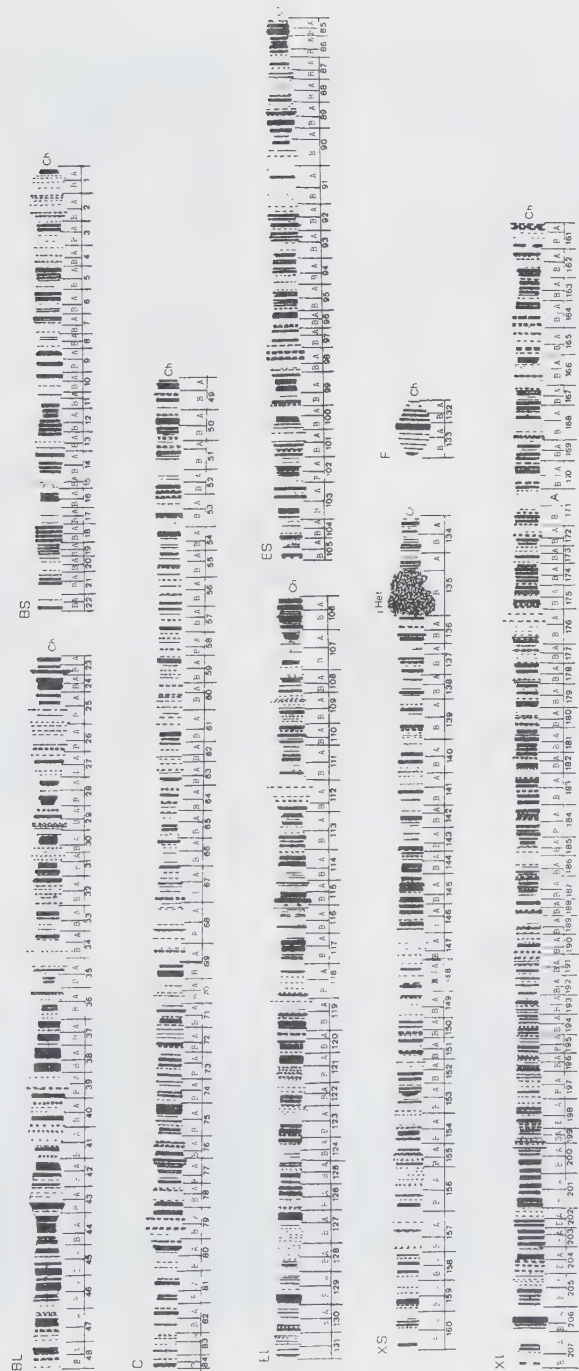
The values are corrected for background.

f: absolute frequency (number of nuclei). n: number of silver grains.

VII. FIGURES

Chromosome Maps of *Drosophila affinis*

Fig 2



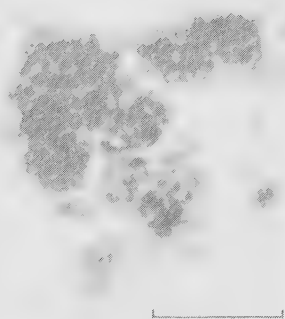


Fig. 1

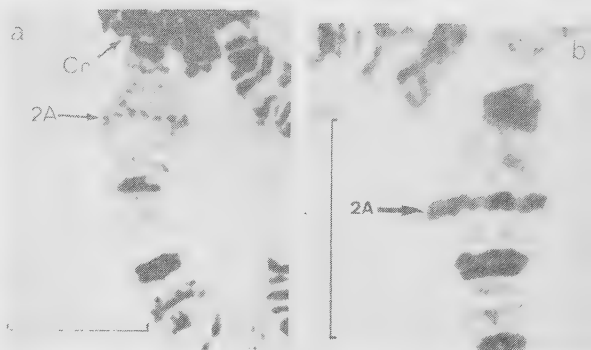


Fig. 3

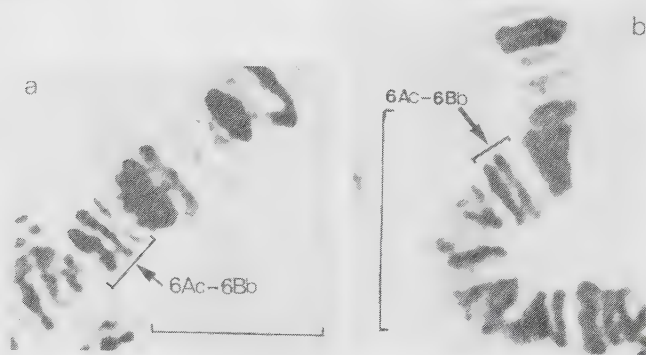


Fig. 4

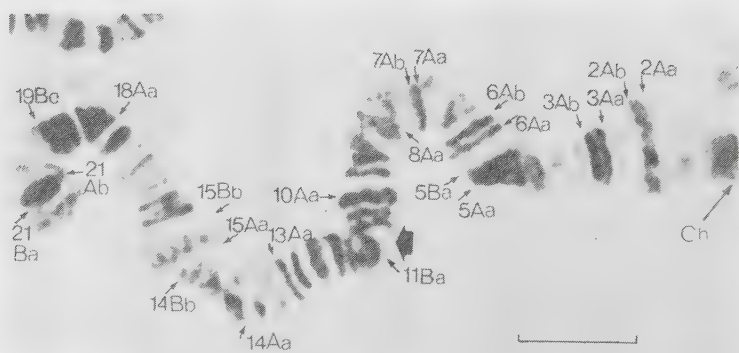


Fig. 5

Fig. 6



Fig. 7

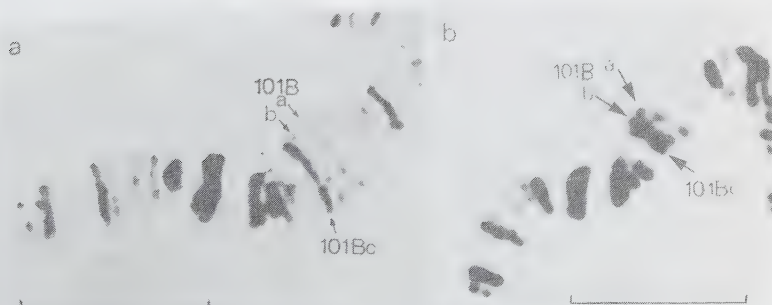
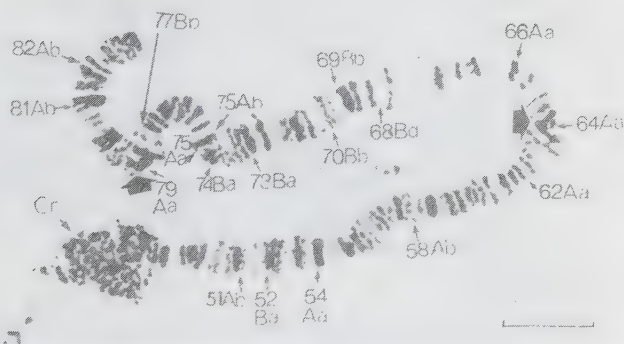


Fig. 8

Fig. 9

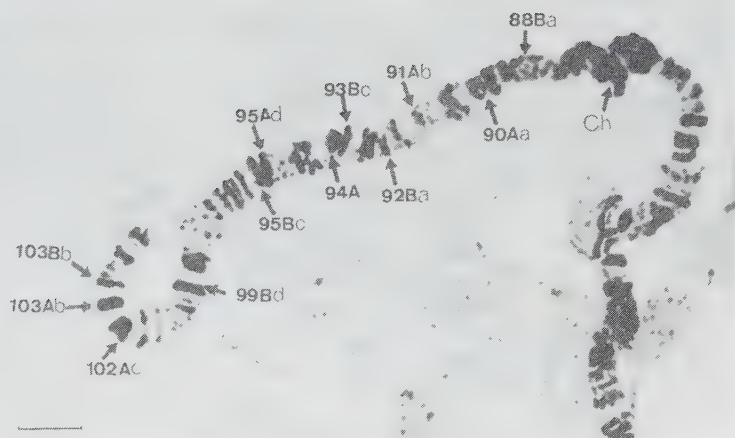


Fig. 10

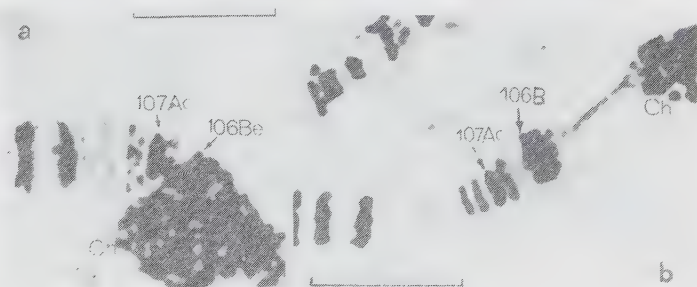


Fig. 11

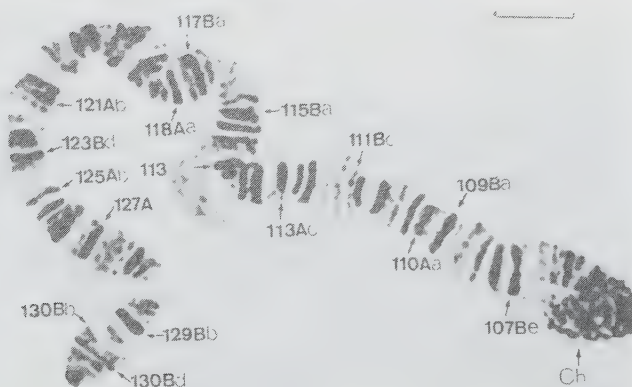


Fig. 12

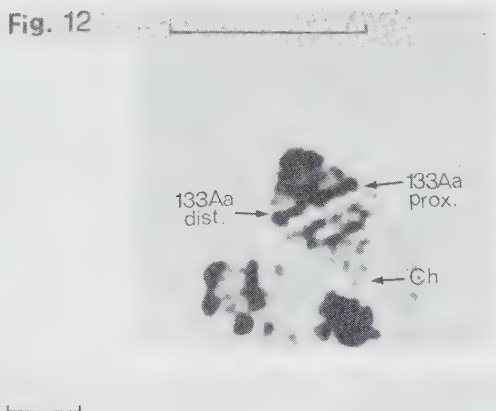


Fig. 13

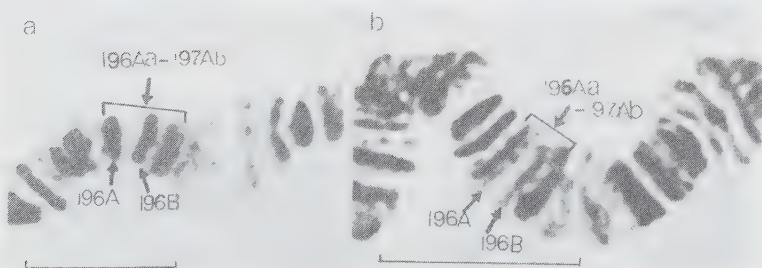


Fig. 14

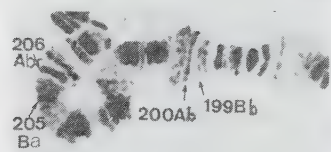
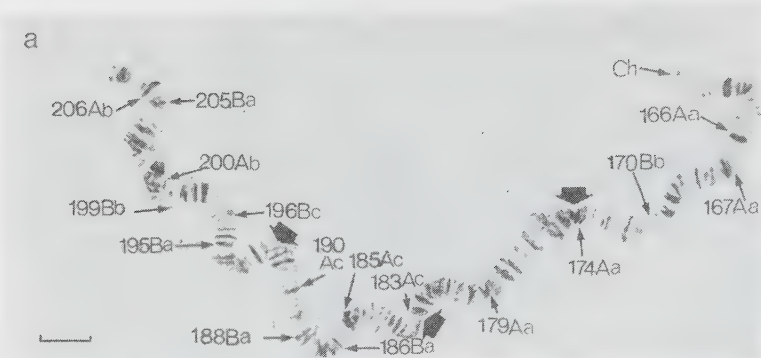


Fig. 15

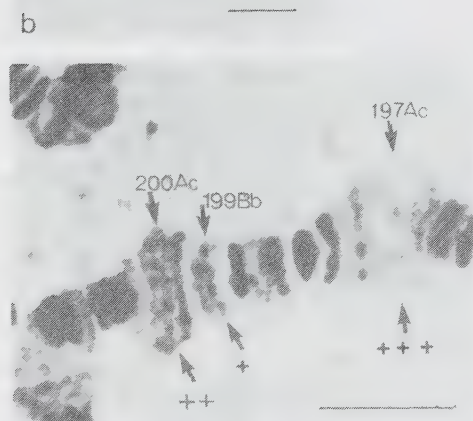


Fig. 16

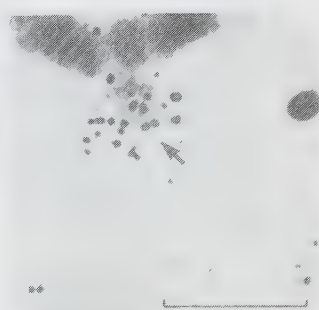


Fig. 17

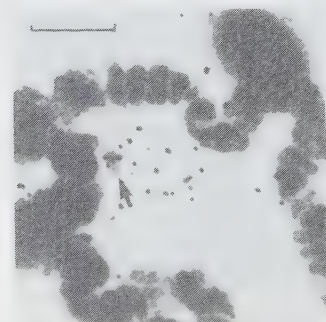


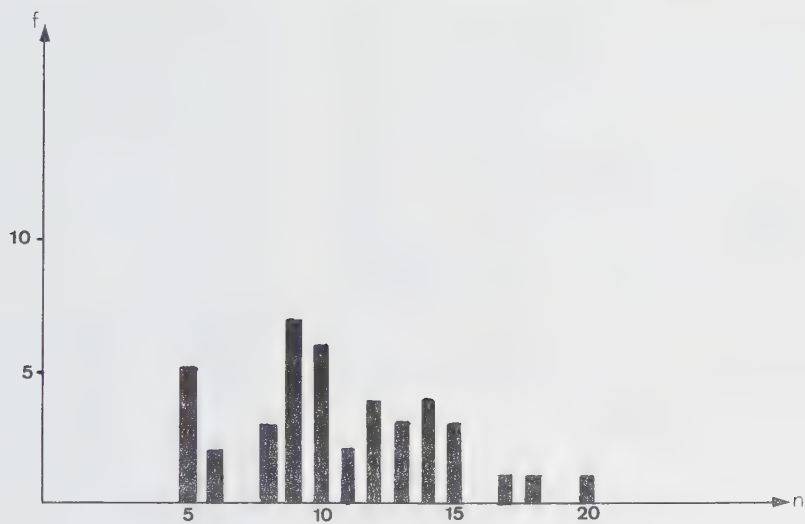
Fig. 19



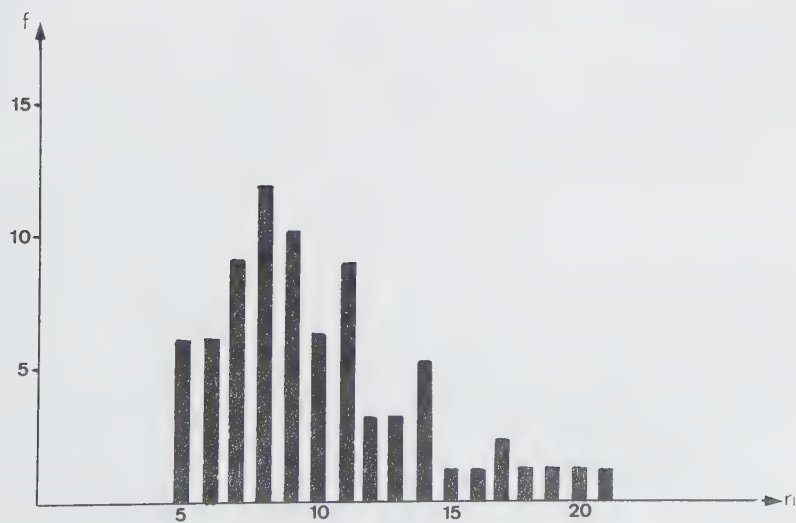
Fig. 20

Fig. 18

a



b



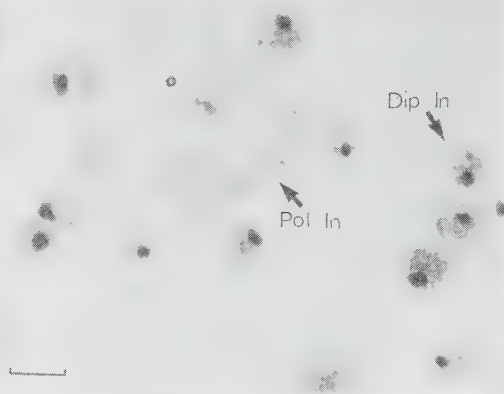


Fig. 21

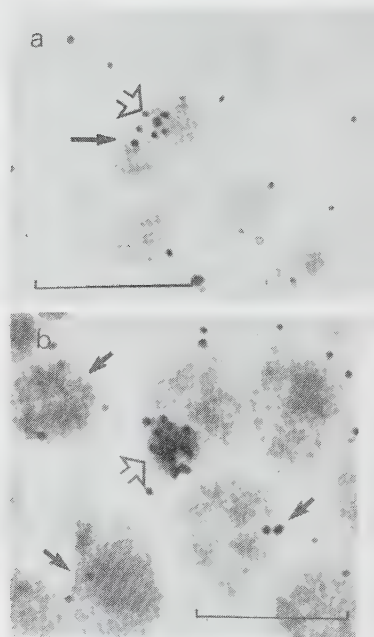


Fig. 22

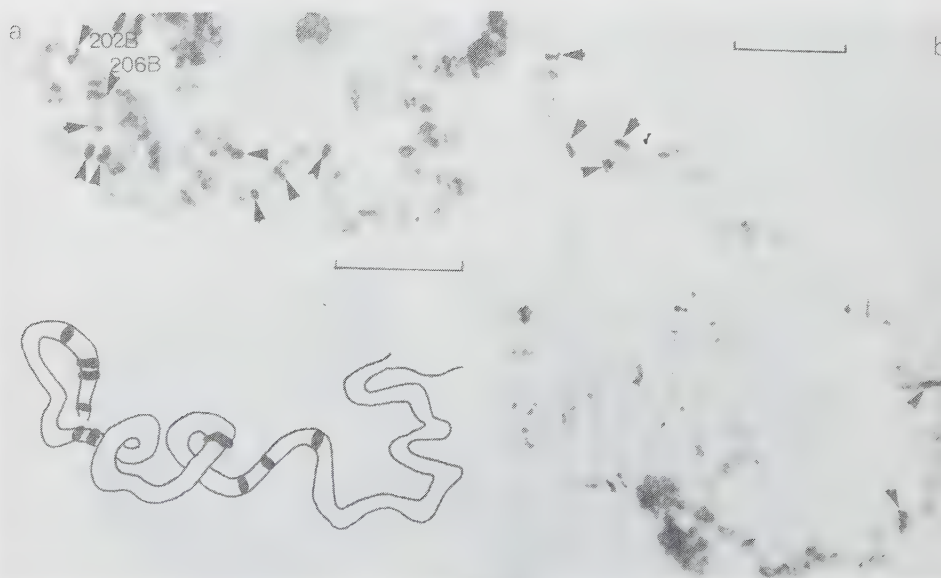
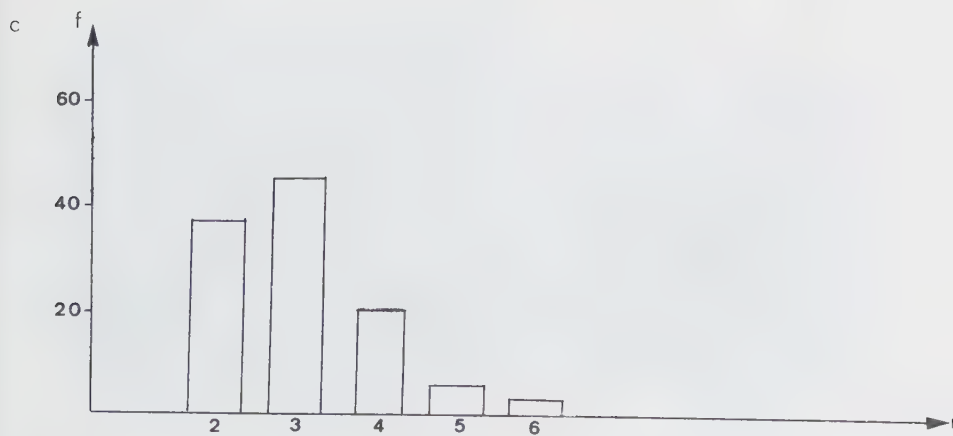
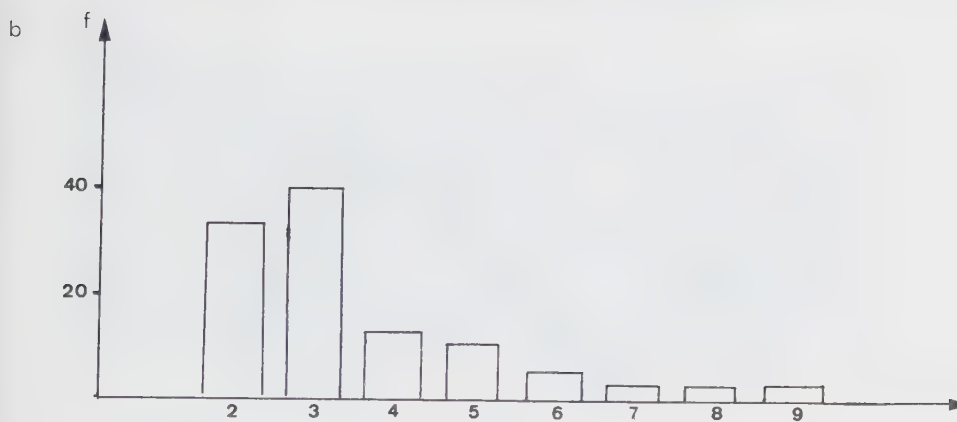
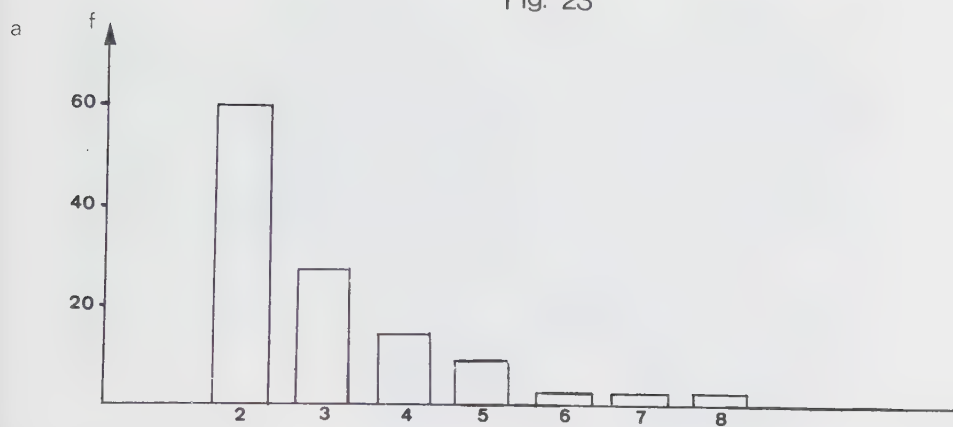


Fig. 24

Fig. 23



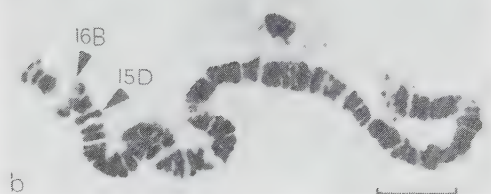
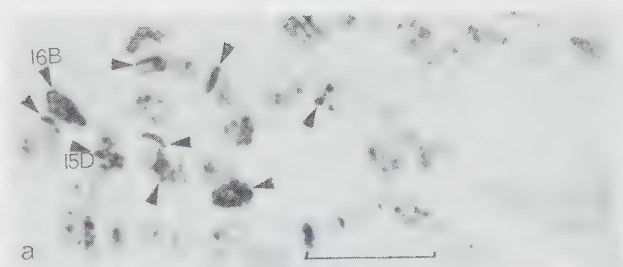
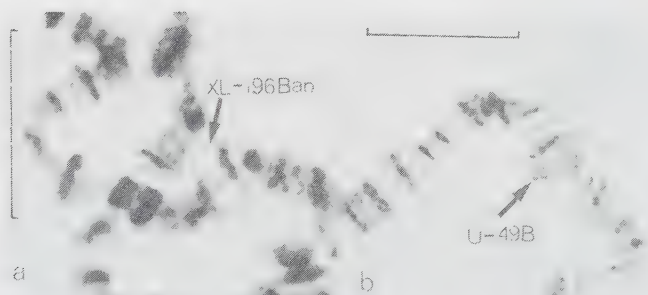


Fig. 25



b

U-49B

Fig. 26

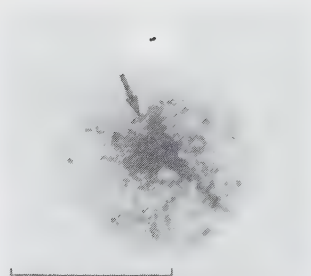


Fig. 27

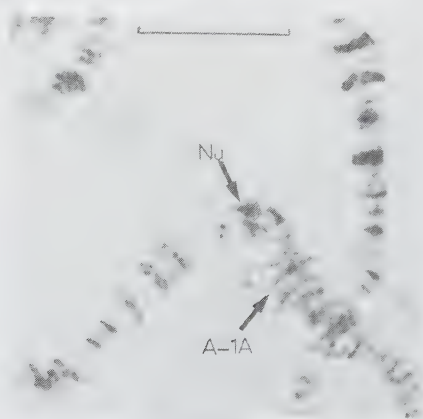


Fig. 28

Fig. 29

Chromosome		1 st	3 ^d	4 th	5 th	2 nd		6 th
						X	Y	(dots)
a	Giemsa C							
b	Ag-AS							

Fig. 30

Chromosome		1 st	2 nd	3 ^d		X	4 th	Y	5 th
									(dots)
a	Giemsa C								
b	Ag-AS								

Fig. 31

Chromosome		2 nd	3 ^d		X	1 st	Y	4 th
								(dots)
a	Giemsa C							
b	Ag-AS							

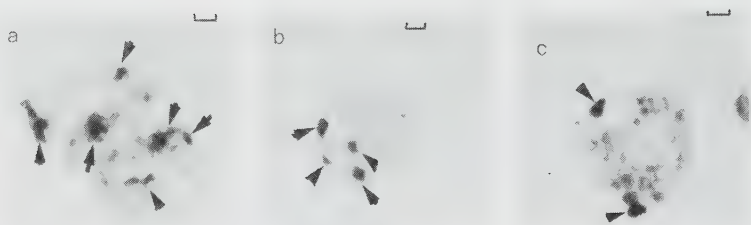


Fig. 32

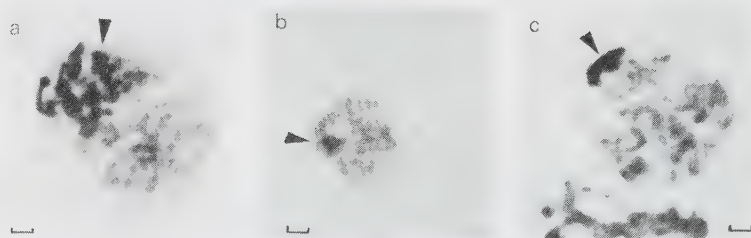


Fig. 33

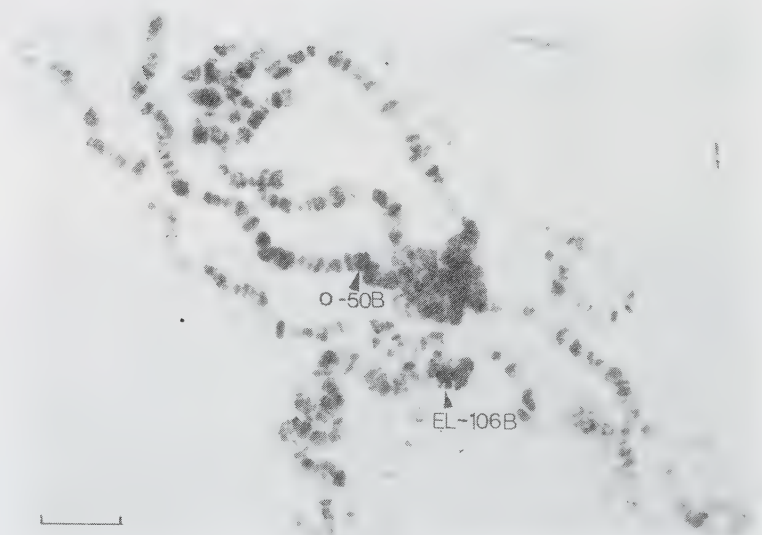


Fig. 35

Fig. 34

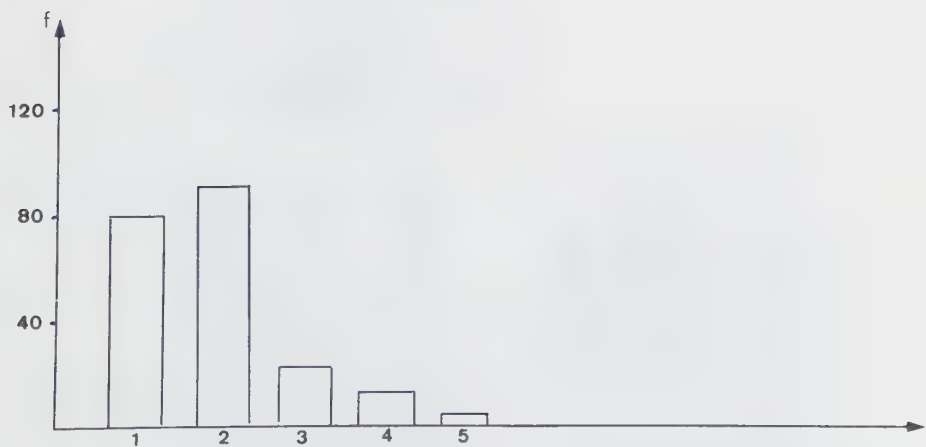
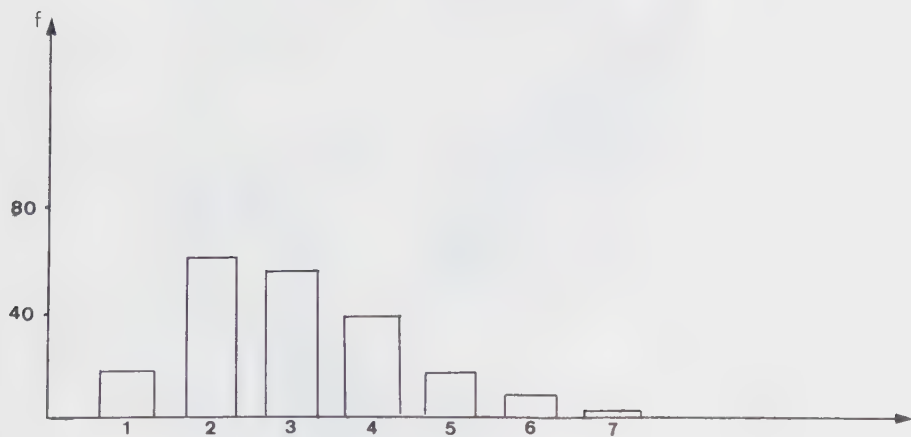
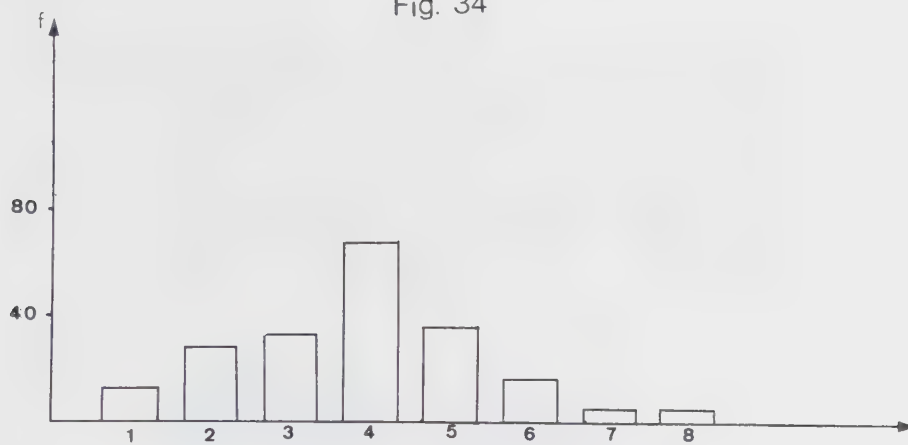
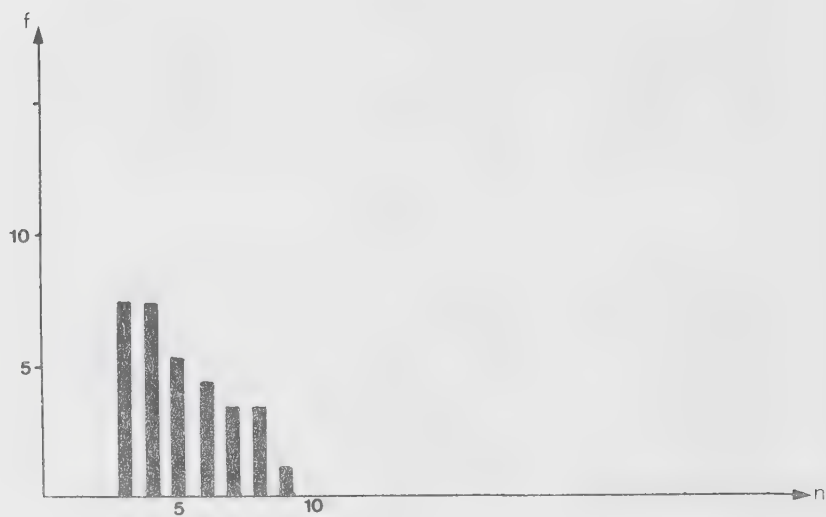


Fig. 36

a



b

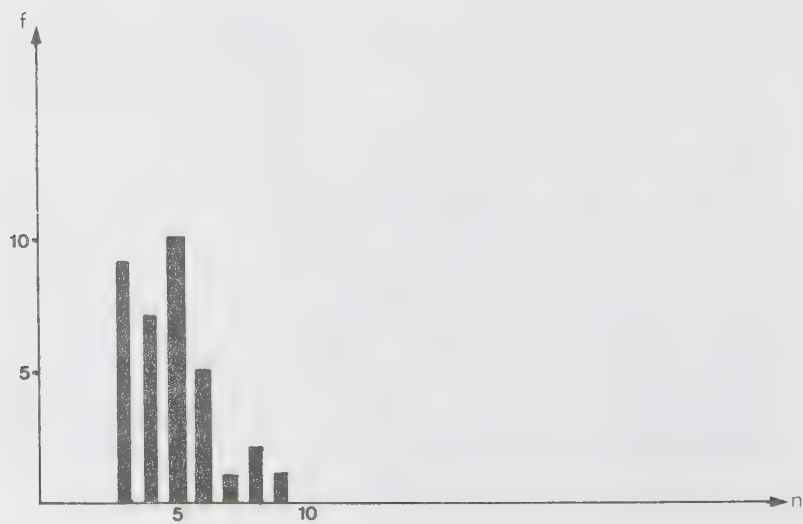


Fig. 37

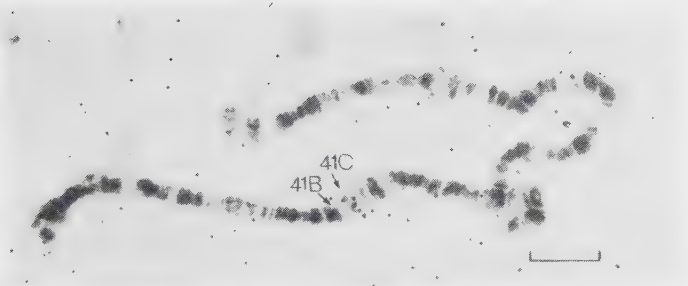


Fig. 38

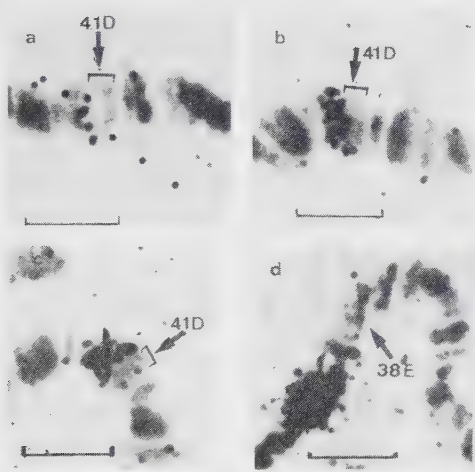


Fig. 39

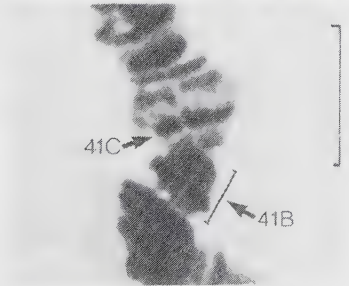


Fig. 40

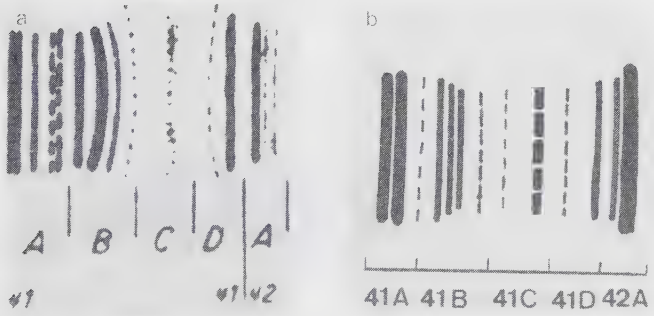
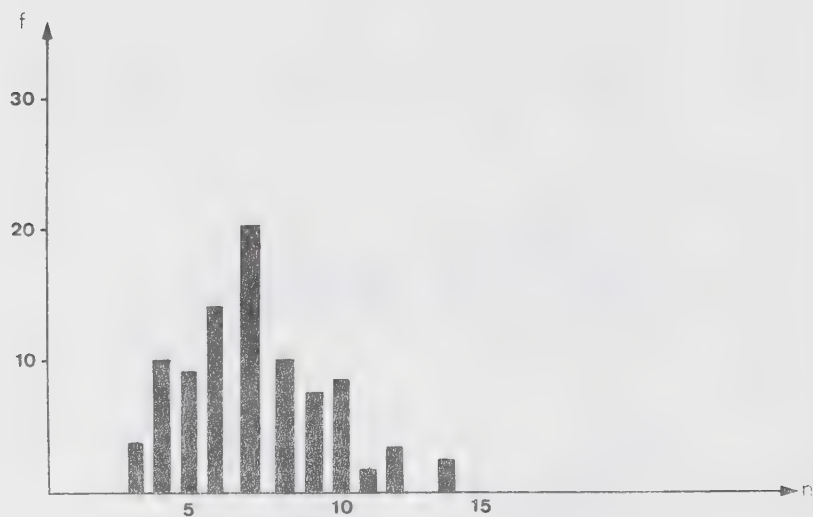
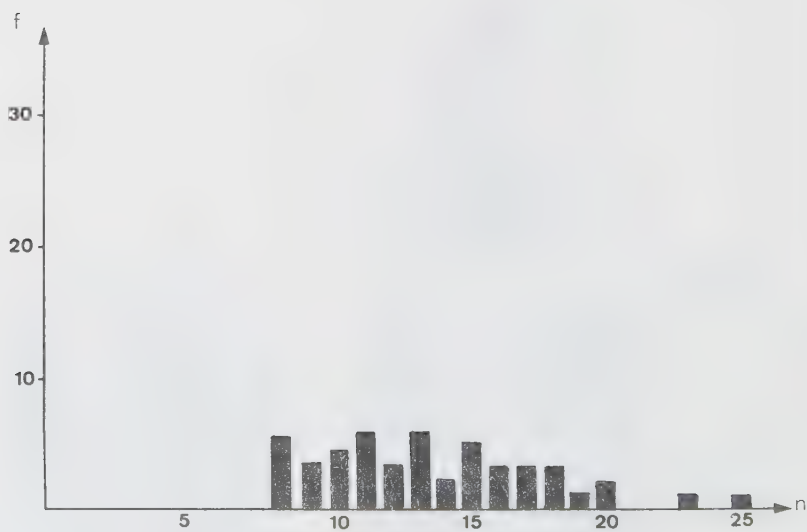


Fig. 41

a



b



VIII. SUMMARY

Histone genes and nucleolus organizing regions (NORs) have been localized in the polytene and mitotic karyotypes of D. affinis and D. subobscura. For histone gene localization, nick-translated tritiated E. coli plasmids with inserted histone sequences from D. melanogaster were in situ hybridised to salivary gland chromosomes of the two species, using the polytene karyotype of D. melanogaster as experimental control for specific hybrid formation. In this species, histone genes have been located to region 39DE (Pardue et al., 1977). In D. affinis, the position of histone genes was detected at two unlinked autosomal sites, regions 50B and 106B, and in D. subobscura at one autosomal site in region 41C. The first complete map of the polytene karyotype in D. affinis is presented, which was used to identify the region comprising the histone genes, while the histone locus in D. subobscura was established according to the salivary gland chromosome map of Kunze-Mühl and Müller (1958).

In revealing the positions of NORs in the mitotic karyotype of the two species, ammoniacal silver-staining (AgAS-technique) indicated their presence on the two dot-like chromosomes, in the centromere-near regions of the acrocentric autosome pair and on the sex chromosomes in neuroblast metaphases of D. affinis. In mitoses of D. subobscura, the sex chromosomes and the first autosome pair of the complement carried NORs. Polytene nuclei exhibited positively silver-staining chromosomal bands in consistent positions throughout the complement in both species, as well as Ag-positive areas within the nucleolus.

These silver-staining nucleolar areas coincide with regions locating rRNA, as it was shown by in situ hybridisation of ^3H -18+28S rRNA extracted from D. melanogaster, whereas in both species polytene nuclei did not exhibit any autoradiographic label over chromosomal bands.

The results are discussed with respect to the effect of particular X-linked gene arrangements, which in both species correlate with a sex ratio phenomenon, in that parental male carriers of the inverted X-chromosome, in crosses with females with any inverted or standard karyotype, give rise to excess female offspring, thus deviating from the expected 1:1 sex ratio.

Abbreviations

A ₂₆₀	absorbance at 260 nm; 1 cm light path
AgAS	Ag ammoniacal silver
bp	base pair
C	Curie-unit = 3.7×10^{10} radioactive trans- formations per second
cpm	counts per minute
dATP	deoxyadenosine triphosphate
dCTP	deoxycytosine triphosphate
dGTP	deoxyguanosine triphosphate
DNA	deoxyribonucleic acid
DNAase I	deoxyribonuclease I
DTT	dithiothreitol
E. coli	Escherichia coli
EDTA	ethylenediaminetetraacetic acid
H	histone fraction
kbp	kilobase pair = 1000 base pairs
mRNA	messenger ribonucleic acid
NOR	nucleolus organizing region
oligo-dT	oligo-deoxythymidine
pDmh	plasmid with DNA-sequences coding for histones of <i>Drosophila melanogaster</i>
polyA ⁺	polyadenosine
PVS	polyvinylsulfate

Abbreviations (cont.)

RNA	ribonucleic acid
RNAase	ribonuclease
rpm	revolutions per minute
rDNA	ribosomal deoxyribonucleic acid
rRNA	ribosomal ribonucleic acid
S	sedimentation coefficient Svedberg unit = 10^{-13} seconds
SDS	sodium dodecyl sulfate
spec. act.	specific activity of a radioactive probe
sr	sex ratio
1 x SSC	0.15M sodium chloride, 0.015M trisodium citrate
TCA	trichloroacetic acid
Tris	tris (hydroxymethyle) aminomethane
TTP	thymidine triphosphate

Acknowledgements

I wish to express sincere thanks to PD Dr. E. Jungen-Hauschteck for the guidance of my thesis and for helpful discussions. I also wish to thank Prof. Dr. M. Birnstiel for valuable advice in the molecular-biological experimental part of the presented studies, as well as for giving his opinion on the manuscript. I am indepted to Dr. J. Burckhardt and Dr. M.L. Melli for technical advice in the isolation of ³H-18+28S rRNA and in nick-translation of cloned histone genes. My husband, PD DR. D. Schweizer, Vienna, gave me invaluable advice on the application of the AgAS-technique, what I am most grateful for.

Curriculum vitae

Am 17. Januar 1950 wurde ich, Claudia Renate Schweizer-Hess, durch Heirat Bürgerin von Glarus, in Basel geboren. Nach dem Besuch von Primar- und Sekundarschule trat ich im Frühjahr 1965 in das Gymnasium II der Höheren Töcherschule (Abt. IV) in Zürich ein. Hier bestand ich im Herbst 1969 die Matur (Typ. B) und immatrikulierte mich im Wintersemester 1969/70 an der Philosophischen Fakultät II der Universität Zürich. Im Herbst 1974 schloss ich mein Studium mit dem Diplom als Naturwissenschaftler (Hauptfach Zoologie) ab.

Die Dissertation entstand unter der Leitung von Frau PD Dr. E. Jungen-Hauschteck. Im Laufe meines Studiums besuchte ich Vorlesungen und Kurse bei den folgenden Dozenten: Bachofen, Batschelet, Baumann, Biegert, Billeter, Birnstiel, Bolliger, Burla, Chen, Clarkson, Cook, Endress, Eugster, Hadorn, Hediger, Hohl, Hunsperger, Jungen-Hauschteck, Jungen, Keusch, Knüsel, Kubik, Kubli, Kuhn-Schnyder, Kummer, Melli, Nöthiger, Oswald, Rast, Rieber, Rohweder, Schaffner, Schlittler, Schmid, Stüssi, Tardent, Thomas, Tobler, Trümpy, Wanner, Weber, Wehner, Weissmann und Ziswiler.

difo
druck

laubanger 15
8600 bamberg
tel.: 0951/6 4321